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The effects of sodium bicarbonate (NaHCO3) on whole body and isolated skeletal muscle performance

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The work contained within this document has been submitted by the student in partial fulfilment of the requirement of their course and award

The effects of sodium bicarbonate ($NaHCO_3$) on whole body and isolated skeletal muscle performance

By

Matthew F. Higgins

A thesis submitted in partial fulfilment of the University's requirements for the Degree of Doctor of Philosophy

July 2013

Dedication

I would like to dedicate this thesis in its entirety to my late father (James Peter Higgins; 19/05/1942 – 13/08/1989), my late mother (Bridget Una Higgins; 27/05/1942 – 06/11/2005), my sister Kathryn, my brother Simon, my niece Nicole and my partner Heather.

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Finally, in the words attributed to Max Planck:

"...Science cannot solve the ultimate mystery of nature. And that is because, in the last analysis, we ourselves are a part of the mystery that we are trying to solve..."

Research Outputs

Results from this thesis have been presented in the following formats:

Journal Articles

(1) **Higgins, M.F**, Tallis, J., Price, M.J., and James, R.S. (2012) 'The effects of elevated levels of sodium bicarbonate ($NaHCO_3$) on the acute power output and time to fatigue of maximally stimulated mouse soleus and EDL muscles'. *European Journal of Applied Physiology* 113(5), 1331-1341. DOI 10.1007/s00421-012-2557-8 **(chapter 6)**

(2) **Higgins, M.F.,** James, R.S., and Price, M.J. (2013) 'The effects sodium bicarbonate ($NaHCO_3$) ingestion on high intensity cycling capacity'. *Journal of Sports Sciences* DOI 10.1080/02640414.2012.758868 **(chapter 5)**

(3) **Higgins, M.F.,** James, R.S., and Price, M.J. (2013) 'Familiarisation to and reproducibility of cycling at 110% peak power output'. *Journal of Sports Medicine and Physical Fitness* (in production) **(chapter 4)**

Presentations

Oral: **Higgins, M.F.,** James, R.S, & Price, M.J. (2011) 'The effects sodium bicarbonate ($NaHCO_3$) ingestion on high intensity cycling capacity'. *British Association of Sport and Exercise Sciences (BASES) – National Conference, Essex 2011* **(chapter 5)**

Poster: **Higgins, M.F.,** James, R.S, & Price, M.J. (2011) 'Familiarisation to and reproducibility of cycling at 110% peak power output'. *British Association of Sport and Exercise Sciences (BASES) – National Conference, Essex 2011* **(chapter 4)**

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Abbreviations

ACD	Acidosis
AD	Abdominal discomfort
ALK	Alkalosis
ATP	Adenosine tri-phosphate
BE	Base excess
BLa	Blood lactate
$CaCO_3$	Calcium carbonate
CA	Carbonic anhydrase
Cl^-	Chloride ion
CON	Control
EDL	Extensor digitorum longus
ES	Effect size
$g.kg^{-1}$	Grams per kilogram
GF	Gut fullness
H^+	Hydrogen ion
Hb	Haemoglobin
HCO_3^-	Bicarbonate ion
H_2CO_3	Carbonic acid
K^+	Potassium ion
kJ	Kilojoules
kN m^2	Kilonewtons per square metre
La^-	Lactate ion
MCT	Monocarboxylate transporter
mM or $mmol.l^{-1}$	Millimoles per litre
MPO	Mean power output
Na^+	Sodium ion
NaCl	Sodium chloride
NAD^+	Nicotinamide adenine dinucleotide
$NaHCO_3$	Sodium bicarbonate
NH_4Cl	Ammonium chloride
OR	Benefit to harm odds ratio
PCr	Phosphocreatine
PFK	Phosphofructokinase
pH	$-\log(10)\ [H^+]$
pH_i	Intracellular pH
pH_e	Extracellular pH
PLA	Placebo
PPO	Peak power output
PRE	Perceived readiness to exercise
RER	Respiratory exchange ratio
RPE	Ratings of perceived exertion
SD	Standard deviation
SE	Standard error
SOL	Soleus
SWC	Smallest worthwhile change
T_{LIM}	Constant power tests to volitional exhaustion
TWD	Total work done
$\dot{V}_E$	Expired minute ventilation
$\dot{V}O_{2MAX}$	Maximal oxygen uptake
$\dot{V}O_{2PEAK}$	Peak oxygen uptake
W	Watts
$W.kg^{-1}$	Watts per kilogram
W_{PEAK}	Peak power output

Abstract

This thesis examined four key areas considered to contribute to why the efficacy of sodium bicarbonate ($NaHCO_3$) as an ergogenic aid remains equivocal. Firstly, familiarisation to and test re-test reliability of continuous constant load cycling to exhaustion (T_{LIM}) at 110% peak power output (W_{PEAK}) were investigated. Results indicated two trials are required before participants become fully familiarised and reliable data are obtained and that daily biological variation was 6 ± 11% (16 ± 28 s). The primary aim of study two was to determine the most appropriate exercise intensity for future studies in this thesis. A secondary aim was to elucidate why certain participants appear to respond to $NaHCO_3$ ingestion and others do not (Price and Simons 2010, Saunders et al. 2011). Therefore, we evaluated cycling T_{LIM} at 100%, 110% and 120% W_{PEAK} in the same participants. $NaHCO_3$ ingestion increased T_{LIM} by 17% compared to placebo (PLA) at 100% W_{PEAK}. This was due, at least in part, to attenuated localised ratings of perceived exertion (RPE_L). No difference in group level data was observed between treatments at 110% W_{PEAK} or 120% W_{PEAK} although there was marked inter and intra individual variance. Thirdly, in order to evaluate the efficacy of $NaHCO_3$ at a tissue level we examined the effects of $NaHCO_3$ on dynamic isolated muscle performance undergoing cyclical length changes. Acute power output (PO) was on average 7.0% greater for $NaHCO_3$ treated extensor digitorum longus (EDL) muscles and 3.6% greater for $NaHCO_3$ treated soleus (SOL) muscles compared to control (CON). Increases in PO were due to greater force production throughout shortening. Treatment of EDL and SOL did not alter the pattern of fatigue at a group level although similar to study 2 there was marked inter individual variation. Finally, to determine the effects of training status we evaluated the effects of 6 weeks high-intensity cycling training on the efficacy of $NaHCO_3$. Overall, pre-training T_{LIM} was 10% greater with $NaHCO_3$ compared to PLA with a benefit to harm odds ratio of 571. Overall, post-training T_{LIM} was 6% greater with $NaHCO_3$ compared to PLA with a benefit to harm odds ratio of 17. Similar to studies 2 and 3 individual variation was observed. Based on daily biological variation for T_{LIM} of 6% (as determined in study 1) and a

recommended benefit to harm odds ratio threshold of > 66, $NaHCO_3$ improved T_{LIM} before training only. We concluded that 6 weeks high-intensity cycling training reduces the effectiveness of $NaHCO_3$ in previously non-cycling trained males. The change in efficacy is likely due to, at least in part, training induced changes in intracellular buffering capacity.

In summary, $NaHCO_3$ is an effective ergogenic aid for T_{LIM} cycling at 100% W_{PEAK} in non-cycling trained males. This is due, at least in part, to attenuated localised ratings of perceived exertion (RPE_L). In contrast, 6-weeks high-intensity cycling training reduces the efficacy of $NaHCO_3$ for T_{LIM} cycling at 100% W_{PEAK} in previously non-cycling trained males. The change in efficacy is likely due to, at least in part, training induced changes in intracellular buffering capacity. At a skeletal muscle level, $NaHCO_3$ increases acute PO in both predominantly fast (EDL) and predominantly slow (SOL) twitch muscle fibres, due to greater force production throughout shortening.

Chapter 1 – Introduction

During brief high-intensity exercise with a significant glycolytic component there is a concomitant increase of lactate (La^-) and hydrogen (H^+) ions in both working muscle and blood due to the accumulation and subsequent disassociation of lactic acid (Halestrap and Price 1999, Thomas et al. 2005, Philp, MacDonald, and Watt 2005). The La^- quickly combines with another ion, such as Na^+, to produce, for example sodium lactate (NaLa). The accumulation of lactate and/or H^+ has been implicated as a cause of muscle fatigue (Sahlin, Tonkonogi, and Söderlund 1998, Allen, Lamb, and Westerblad 2008), which can be defined as any reduction in capacity to generate force or power output as a result of exercise (Vøllestad 1997). Although the 'lactic acidosis' theory of fatigue still divides academic opinion (Brooks 2001, Cairns 2006, Allen, Lamb, and Westerblad 2008) augmenting the body's ability to neutralise excess H^+ might prolong exercise capacity (Begum, Cunliffe, and Leveritt 2005, Cairns 2006). The primary mechanism in which $NaHCO_3$ is thought to exert ergogenic benefit is by augmenting the bioavailability of bicarbonate ions [HCO_3^-] which combines with H^+ to form carbonic acid (H_2CO_3) which is then (reversibly) converted into CO_2 and H_2O by carbonic anhydrase (Sahlin et al. 1978, Robergs 2002, Peart, Siegler, and Vince 2012). The CO_2 is expelled through ventilation and H_2O exists most likely as metabolic water. Indeed, [HCO_3^-] is arguably the most important extracellular buffer (McNaughton, Siegler, and Midgley 2008, Poupin et al. 2012). Therefore, by undertaking exogenous supplementation of $NaHCO_3$, several researchers have postulated that this might provide the body with added fatigue resistance during exercise against H^+ induced disruptions in acid-base homeostasis (Lavender and Bird 1989, McNaughton 1992a,b, McNaughton, Ford, and Newbold 1997, McNaughton, Dalton, and Palmer 1999, Cameron et al. 2010, Price and Simons 2010).

Despite the proposed biochemical basis for augmented H^+ buffering with $NaHCO_3$ supplementation empirical research has delivered varied results (Matson and Tran 1993, Requena et al. 2005, McNaughton, Siegler, and Midgley 2008, Price and Simons 2010,

Peart, Siegler, and Vince 2012). The variable results in research evaluating the efficacy of $NaHCO_3$ on exercise performance / capacity are due, at least in part, to variations in the dosage administered, degree of metabolic alkalosis induced, the intensity, duration and nature of the exercise undertaken, participant training status and pre-experimental procedures (Matson and Tran 1993, Maughan 1999, McNaughton, Siegler, and Midgley 2008, Peart, Siegler, and Vince 2012). Although the first (0.3 $g.kg^{-1}$ body mass; McNaughton 1992a) and second (~ 60/90 mins pre-exercise; Renfree 2007, Price and Singh 2008) aspects have been addressed, the effects of $NaHCO_3$ on exercise capacity over a range of exercise intensities within the same population have yet to be confirmed. By undertaking this study the following objectives will be achieved: (1) provide further evidence to the efficacy (or not) of $NaHCO_3$ as an ergogenic aid, (2) evaluate the perceptual, physiological and biochemical responses to exercise after $NaHCO_3$ ingestion in the same participants at different exercise intensities and (3) confirm whether individuals can be classified as either responders or non-responders to $NaHCO_3$ (Price and Simons 2010, Saunders et al. 2011). Such evidence is likely to further elucidate how $NaHCO_3$ affects exercise capacity (Price and Simons 2010).

The wide variation in participant training status (both between and within studies) might also affect results in the area of $NaHCO_3$ research. Aschenbach et al. (2000) suggested the highly trained wrestlers in their study might already possess a high intracellular buffering capacity which left little opportunity for enhanced extracellular buffering, such as through $NaHCO_3$ ingestion, to be effective. However, it should be acknowledged that an ergogenic benefit following $NaHCO_3$ supplementation has been reported in highly trained runners (McNaughton and Davies 1988, Bird, Wiles, and Robbins 1995) and cyclists (Driller et al. 2012ab). Despite an individual's training status potentially affecting responses to $NaHCO_3$ ingestion (Linderman et al. 1992, Aschenbach et al. 2000, Peart, Siegler, and Vince 2012) and a number of studies having examined the effects of $NaHCO_3$ ingestion *prior* to high-intensity training on a variety of physiological and

performance parameters (Edge, Bishop, and Goodman 2006, Thomas et al. 2007, Bishop et al. 2010), no research has examined how an acute change in training status might affect the efficacy of $NaHCO_3$. Indeed, a recent meta-analysis suggests that untrained individuals are far more likely to observe ergogenic benefit than trained individuals (Peart, Siegler, and Vince 2012). Therefore, in evaluating the efficacy of $NaHCO_3$ pre and post high-intensity training, the following three objectives will be achieved: (1) further examine the efficacy of $NaHCO_3$ in healthy but non cycling trained males (2) examine the effects of 6 weeks high-intensity cycling training on healthy but non cycling trained males and (3) examine how an acute change in training status affects the efficacy of $NaHCO_3$. This evidence will help further elucidate how $NaHCO_3$ affects exercise capacity.

The ability to detect changes outside of day-to-day variation in exercise tests (i.e. sensitivity) is of particular importance in research where interventions such as nutritional supplementation are evaluated (Sewell and McGregor 2008). To minimise systematic bias, participants are often familiarised with the proposed tests before collecting performance data (Lavender and Bird 1989, Carey and Richardson 2003) and in performing enough such trials, learning effects or other systematic changes are diminished sufficiently so that reliable data can be obtained (Hopkins 2000). However, there is little consistency in terms of pre-experimental familiarisation within the literature evaluating the efficacy of $NaHCO_3$ on exercise performance / capacity. Moreover, despite constant power tests to exhaustion (T_{LIM}) being the most reliable physical performance test (Hopkins, Schabort, and Hawley 2001) there is a paucity of research evaluating their reliability (Morris et al. 2011). At the time of commencing this research (January 2010) no research had evaluated the reliability of T_{LIM} using the minimum of 3 trials as recommended by Hopkins (2000). However, the recent publication by Saunders et al. (2012) who addressed the reliability of T_{LIM} at 110% W_{MAX} is acknowledged. The results of the Saunders et al. (2012) study in relation to the similar work presented here are discussed in greater detail in study 1 (chapter 4). In establishing the reliability of the T_{LIM} cycling protocol, the following three objectives will be achieved: (1)

provide reliability data on cycling T_{LIM} thus ensuring participants are optimally familiarised for the studies contained within this thesis, (2) establish the daily biological variation of healthy non cycling males undertaking T_{LIM} cycling exercise in our laboratory and (3) assess whether the lack of consistency in pre-experimental procedures in previous research might have contributed to the equivocal results related to the efficacy of $NaHCO_3$ as an ergogenic aid.

In an attempt to elucidate the effects of modulating acid-base balance at a tissue level several studies have examined the effects of metabolic acidosis and alkalosis on isolated muscle performance. Spriet et al. (1985) induced metabolic acidosis by lowering the [HCO_3^-] in the isolated muscle perfusate from ~ 24 mM to ~ 13 mM. Metabolic acidosis significantly increased the rate of muscle tension decay and reduced absolute muscle tension in the gastrocnemius-plantaris-soleus muscle group in rats, during fatiguing isometric stimulation, when compared to CON. Conversely, Spriet et al. (1986) found that inducing metabolic alkalosis by increasing [HCO_3^-] from ~ 21 mM to ~ 27 mM had no effect on peak isometric tension or tension decay compared to CON. Finally, Broch-Lips et al. (2007) examined the effect of 40 mM and 25 mM [HCO_3^-] on isometric force production in isolated rat skeletal muscle. The elevated [HCO_3^-] had no significant effect on force maintenance during continuous stimulation or recovery of force during brief tetanic stimulation in soleus or on tetanic force development in extensor digitorum longus muscles at 30°C. Similarly, 40 mM of HCO_3^- had no significant effect on isometric force maintenance during either continuous stimulation or intermittent stimulation protocols (1 s on, 3 s off) at 37°C (Broch-Lips et al. 2007).

Although the aforementioned *in vitro* studies have examined the effects of high and low [HCO_3^-] on muscle performance the current body of isolated muscle research has a number of methodological concerns. For example, during mammalian locomotion muscles that are attached to moving skeletal structures, either directly or indirectly, undergo repetitive length changes (Josephson 1993). Approximation of such length changes *in vitro* facilitates

the evaluation of important components of exercise performance such as recovery from fatigue, (James, Wilson, and Askew 2004) as well the possible direct effects of ergogenic aids (Tallis et al. 2012) in mammalian muscle. As such, research using isometric muscle protocols has limited application to muscle performance during dynamic exercise which is exhibited in most mammalian locomotion. Furthermore, no research to date examining acid-base balance at a tissue level has used concentrations of HCO_3^- that are typically achieved in the blood of human participants (~ 32 mM; Kolkhorst et al. 2004, Price and Singh 2008, Lindh et al. 2008, Siegler et al. 2010) following the recommended supplementation dosage (0.3 $g.kg^{-1}$; McNaughton 1992a). Moreover, there is a paucity of research that provides ecologically valid links between *in vivo* and *in vitro* exercise performance. Therefore, *in vitro* research that addresses these key gaps would provide useful data as to how augmented [HCO_3^-] might affect human exercise performance / capacity.

In summary, the main aim of this thesis is to examine the effects of sodium bicarbonate ($NaHCO_3$) on whole body and isolated skeletal muscle performance. This will be achieved by meeting the following specific objectives:

- To establish the reliability of a suitable exercise protocol to use in the evaluation of oral $NaHCO_3$ ingestion on human exercise performance
- To evaluate the effects of $NaHCO_3$ ingestion on exercise capacity over a range of exercise intensities in the same participants
- To evaluate the effects of elevated levels of $NaHCO_3$ on dynamic isolated muscle performance undergoing cyclical length changes
- To evaluate how a change in training status might affect the efficacy of $NaHCO_3$ ingestion as an ergogenic aid

Chapter 2 – Literature Review

2.1 Introduction

Skeletal muscle fatigue as a result of high-intensity exercise is extremely complex, multi-factorial and is still not fully understood (Noakes 2000, Begum, Cunliffe, and Leveritt 2005, Allen, Lamb, and Westerblad 2008, Artioli et al. 2010, Debold 2012). Furthermore, the numerous possible contributory factors are likely to vary and interact differently between individuals based on genetics, environmental conditions, training status / methods and dietary manipulation. However, based on the premise of this thesis, only fatigue based on specific metabolite accumulation will be examined (Figure 2.1). As such, the remaining content will focus on how $NaHCO_3$ might attenuate exercise induced perturbations in acid-base homeostasis.

Figure 2.1 Possible sites/mechanisms of fatigue during high-intensity exercise (Begum, Cunliffe, and Leveritt 2005)

To provide suitable context to the issues addressed by this the physiological and biochemical basis of acid-base homeostasis will be examined. Secondly, a review of the current literature related to the use of $NaHCO_3$ and its' effects on whole body and isolated skeletal muscle performance will be presented. With respect to whole body research (i.e. in humans) key areas for analysis will evaluate how variations in exercise duration, administration dosage / timing / method, loading regime, participant training status and exercise modality might influence the efficacy of $NaHCO_3$ as an ergogenic aid. With respect to exercise modality, there will be a predominant focus on cycling as this is the exercise modality adopted within this thesis. However, a variety of other exercise modalities will also be analysed, albeit in less detail, to provide a fuller picture of the efficacy of $NaHCO_3$ on exercise capacity and/or performance. The literature related to the impact of $NaHCO_3$ on isolated muscle performance will consider, amongst others aspects variations in administration dosage and experimental protocol.

2.1.1 Acid-Base Homeostasis

Acid-base homeostasis refers to the physiological and biochemical processes that take place in both humans and animals which aim to achieve an appropriate biological environment for optimal function and acid-base homeostasis is a vital function of living organisms (Adrogué and Adrogué 2001, Poupin et al. 2012). The status of this biological environment results from the balance between acids and bases that are produced / eliminated during biological functions. An acid is defined as any compound which forms hydrogen ions (H^+) in solution and is often referred to as a proton donor. A base is defined as a compound that combines with H^+ in solution and thus is often referred to as a proton acceptor (Drage and Wilkinson 2001). The difference in quantity between acids and bases within the body (i.e. the modulation of H^+) is commonly referred to as acid-base balance (McNamara and Worthley 2001).

2.1.1.i The Hydrogen Ion and pH

The hydrogen ion is a single positively charged particle, the proton (H^+), which is not orbited by any electrons and as such is the smallest ionic particle, and highly reactive. The pH scale was developed in order to simplify the quantification of H^+ and is calculated as the negative logarithm of the H^+ concentration. Due to the logarithmic nature of pH a change of one unit in pH represents a tenfold change in H^+ concentration (Drage and Wilkinson 2001, Germann and Stanfield 2005; Equation 1).

$$pH = - \log(10) [H^+] \qquad \text{(Eq. 1)}$$

Equation 1 Formula for calculation of pH; $[H^+]$ is the hydrogen ion concentration

The physiological range for pH in blood plasma is 7.35 to 7.45 and for arterial blood 7.38 to 7.42. Deviations of pH less than 7.35 and more than 7.45 are known as acidosis and alkalosis, respectively. Maintaining pH within the appropriate optimal range is crucial as deviations can cause deleterious effects to important physiological proteins such as enzymes. Moreover, acidosis can cause a decrease in the excitability of neurons, especially in the central nervous system (CNS) as well as cardiac arrhythmias and vasodilation of blood vessels to the skin due to impaired activity of catecholamines (Drage and Wilkinson 2001, Germann and Stanfield 2005).

2.1.1.ii Sources of perturbations of acid-base balance

Dietary intake can profoundly affect acid-base balance (Greenhaff, Gleeson, and Maughan 1988a,b) in the form of H^+ from amino acids and fatty acids. Metabolic disturbances in acid-base balance can also be due to alterations in renal function, severe vomiting or diarrhoea. Additionally, during cellular metabolism a number of acids are

produced such as lactic acid and ketoacids (Germann and Stanfield 2005). However, it is the potentially acidotic metabolites (H^+ / lactic acid) as a result of high-intensity exercise with which this thesis is primarily concerned.

2.1.1.iii Defence against perturbations of acid-base balance

Three key mechanisms defend the body against deleterious changes in acid-base balance that occur during high-intensity exercise. They are: (1) intracellular and extracellular buffers, (2) respiratory compensation and (3) renal compensation (McNamara and Worthley 2001). Intracellular and extracellular buffering are biochemical processes which provide an immediate response to disturbances in acid-base balance. However, respiratory and renal compensation are slower reacting physiological processes which take minutes (respiratory), hours or even days (renal) to correct an acid-base disturbance. For example, within minutes of a decrease in blood pH, peripheral chemoreceptors activate an increase in alveolar ventilation thereby reducing pCO_2. Given that CO_2 is in equilibrium with H^+ and HCO_3^-, the law of mass action dictates that H^+ and HCO_3^- are reduced and as a consequence blood pH is elevated. If the acid-base disturbance is more chronic, perhaps through severe vomiting or diarrhoea, the renal system can compensate for changes in pH. The kidneys regulate decreases/increases in pH by increasing/decreasing H^+ secretion and increasing/decreasing HCO_3^- reabsorption, respectively. The kidneys can also synthesise new HCO_3^- if the coupling process of increasing H^+ secretion and HCO_3^- reabsorption are insufficient to regulate reductions in pH (Germann and Stanfield 2005). Although it is acknowledged that all three play important biological roles, due to this thesis focussing on relatively short duration high-intensity exercise only intracellular and extracellular buffering will be discussed in the following section.

2.2 Acid-base buffering

An acid-base buffer is a compound that minimises changes in pH when an acid or base is added to, or removed from, a solution (McNamara and Worthley 2001) and is the initial defence against changes in pH (Adrogué and Adrogué 2001). The three main physiochemical buffers are: (1) bicarbonate ions [HCO_3^-], (2) proteins and phosphates and (3) haemoglobin. Because a buffer must be able to release and bind H^+ it is both an acid and a base (Drage and Wilkinson 2001, Germann and Stanfield 2005).

2.2.1 Bicarbonate [HCO_3^-]

The bicarbonate/carbonic acid [HCO_3^- / H_2CO_3] system is regarded as the major buffering system in the body (Cordat and Casey 2009, Poupin et al. 2012). This system functions in both the intracellular and extracellular fluid through the reversible reaction displayed in equation 2:

$$HCO_3^- + H^+ \longleftrightarrow H_2CO_3 \xleftrightarrow[\text{Carbonic Anhydrase}]{} CO_2 + H_2O \longleftrightarrow CO_2 \qquad \text{(Eq. 2)}$$

Equation 2 Interaction between HCO_3^- and H^+ in acid-base buffering (Robergs 2002)

The [HCO_3^- / H_2CO_3] system functions with a pK' of ~ 7.4, where pK' refers to the pH at which half of the acid molecules are deprotonated (Robergs 2001). In other words this is the pH when there is equilibrium between the H^+ that leaves and the H^+ that re-attach to the acid functional group of the molecule. Strong acids have a pK' much lower than ~ pH 7.0 and weak acids have a pK' much closer to ~ pH 7.0 (Robergs 2001, 2002). However, because [HCO_3^-] in isolation has a pK' of 10.2 and [H_2CO_3] has a pK' of 3.77 there is often confusion as to why the bicarbonate system is the body's main blood buffer at physiological pH (Robergs 2002). Equation 2 is often shortened by exercise physiologists and in doing so fails to highlight the full range of reactions that take place that facilitate maintenance of acid-base homeostasis (i.e. pK' of ~ 7.4). The following three reaction constants ($K_{1, 2, 3}$) are key

to the [HCO_3^- / H_2CO_3] system as described in equation 2, with the latter seemingly most unacknowledged in the exercise physiology literature. Most importantly it is the combination of $K_{1, 2, 3}$ which raises the pK' of this buffering system to ~ 7.4 making it very effective within the physiological pH range (Robergs 2002).

$K_1 = [H^+] [HCO_3^-] / [H_2CO_3]$

$K_2 = [H_2CO_3] / [CO_2d] [H_2O]$

$K_3 = [CO_2d] / [CO_2g]$

Where K_N = reaction constant; CO_2d = dissolved CO_2 and CO_2g = gaseous CO_2

The active centres of the sarcolemma bound carbonic anhydrase (CA) isoforms (CAIV and CAXIV) are orientated towards the extracellular space. In accelerating the hydration/dehydration reaction outlined in equation 2, CAIV and CAXIV play crucial roles in maintaining sufficient release of H^+ and lactate from skeletal muscle (Messonier et al. 2007) presumably in conjunction with monocarboxylate transporters (MCTs). Moreover cytosolic isoforms of CA (CAII and CAIII) might also play an important role in muscle pH and muscle lactate regulation during high-intensity exercise (Messonier et al. 2007).

2.2.2 Proteins and phosphates

Due to the amine (NH_2) and carboxylic acid (COOH) groups, proteins also function as acid-base buffers. Owing to the relative alkalinity of blood plasma, proteins exist in their anionic form and act as bases by binding excess H^+ (Equation 3). Examples of proteins that contribute to intracellular buffering capacity are histidine residues of proteins, free histidine and dipeptides such as carnosine and anserine (Begum, Cunliffe, and Leveritt 2005).

$$NH_2\text{-protein-}COO^- + H^+ \longleftrightarrow NH_2\text{-protein-COOH} \qquad \text{(Eq. 3)}$$

Equation 3 Buffering of H^+ by anionic proteins (Poupin et al. 2012)

Further excess H^+ can also be added to an uncharged protein (Equation 4). Since both buffering reactions with proteins are reversible, proteins can also act as acids by releasing H^+.

$$NH_2\text{-protein-COOH} + H^+ \longleftrightarrow NH_4^+\text{-protein-COOH} \quad \text{(Eq. 4)}$$

Equation 4 Buffering of H^+ by uncharged proteins (Poupin et al. 2012)

Disodium hydrogen phosphate (Na_2HPO_4) and hydrogen phosphate (HPO_4^{2-}) can also remove excess H^+. This is in the form of urinary phosphate ($H_2PO_4^-$) and can be demonstrated through the following reactions (Equations 5 and 6):

$$Na_2HPO_4 \longleftrightarrow HPO_4^{2-} + 2Na^+ \quad \text{(Eq. 5)}$$

$$HPO_4^{2-} + H^+ \longleftrightarrow H_2PO_4^- \quad \text{(Eq. 6)}$$

Equations 5 and 6 Buffering of H^+ with disodium hydrogen phosphate (Na_2HPO_4) and hydrogen phosphate (HPO_4^{2-}; Poupin et al. 2012)

2.2.3 Haemoglobin

As haemoglobin (Hb) can bind to both CO_2 and H^+ it has a powerful utility in the modulation of acid-base balance. As deoxygenated Hb has the greatest affinity for both CO_2 and H^+ the most powerful buffering with Hb occurs in the intracellular space (i.e. tissues). Dissolved CO_2 passes into red blood cells down its concentration gradient where it combines with water to form carbonic acid (Equation 2). Subsequently H^+ binds to reduced Hb to form HHb with the HCO_3^- generated by this process being transported back into the plasma in exchange for chloride ions (Cl^-; Equation 7). This exchange promotes equilibrium in that there is no net loss or gain of negative ions in red blood cells. In the lungs this process is

reversed and HHb recombines with HCO_3^- to form CO_2 which passes into the alveoli. The reduced Hb is reformed to return to the tissues (Drage and Wilkinson 2001).

$$H_2CO_3 \longleftrightarrow HCO_3^- + H^+ + Hb \longleftrightarrow HHb + [HCO_3^- \longleftrightarrow Cl^-] \quad \text{(Eq. 7)}$$

Equation 7 Buffering of H^+ with haemoglobin (Hb; Drage and Wilkinson 2001).

2.2.4 Fractional contribution of buffers

Although the [HCO_3^- / H_2CO_3] system is regarded as the major buffering system in the body (Drage and Wilkinson 2001, Poupin et al. 2012) the fractional contribution within the intracellular and extracellular environments differ. The [HCO_3^- / H_2CO_3] system accounts for 36% of intracellular fluid buffering with the remaining 64% buffered by proteins/phosphates. In contrast, the [HCO_3^- / H_2CO_3] system accounts for 86% of extracellular fluid buffering, the remaining 14% by proteins/phosphates (Adrogué and Adrogué 2001, Poupin et al. 2012). Similarly large values for HCO_3^- buffering have been observed during exercise in healthy male participants. Beaver, Wasserman and Whipp (1986) reported that during incremental exercise to exhaustion 92% of H^+ is buffered by HCO_3^- although the proposed split between intracellular and extracellular buffering of H^+ is not reported.

2.2.5 Monocarboxylate transporters (MCTs)

Monocarboxylates are pivotal to cellular metabolism and central to their importance are their rapid translocation across the plasma membrane facilitated by a specific range of proton-linked monocarboxylate transporters (MCTs; Halestrap and Price 1999). According to Halestrap and Meredith (2004) only the first four (MCT1–MCT4) have been demonstrated experimentally to precipitate the proton-linked transport of metabolically important monocarboxylates such as lactate, pyruvate and ketone bodies. Of particular importance

during high-intensity exercise is the facilitated diffusion of lactate with a proton (H^+) across the plasma membrane. Indeed, if efflux of lactic acid does not match production cytosolic pH can be compromised by increased intracellular concentrations of H^+. This can lead to inhibition of important glycolytic enzymes such as phosphofructokinase (PFK; Halestrap and Price 1999).

MCT1 is especially prevalent in cardiac and oxidative (red) skeletal muscle (Halestrap and Price 1999, Halestrap and Meredith 2004) but is universally expressed. According to Halestrap and Price (1999) MCT1 is up-regulated in response to increased work and therefore plays an important role in lactic acid oxidation. In contrast, MCT4 is dominant where lactic acid efflux is high and is therefore most prevalent in white muscle and other cells with a high glycolytic rate. As such both MCT1 and MCT4 play important roles during high-intensity exercise (Halestrap and Price 1999). Indeed, Thomas et al. (2005) found that endurance trained participants presented with 44% greater MCT1 than less trained participants. Moreover, MCT1 expression was negatively correlated ($r = -0.56$, $P < 0.05$) with blood lactate after supramaximal exercise suggesting MCT1 might augment tolerance to fatigue. Although MCT4 was not reported to be statistically different between groups, MCT4 was 29% higher in trained individuals. This difference also demonstrated an extremely large effect size (not published) of 2.5 for differences in MCT4 (3.5 for MCT1) suggesting differences in MCT4 might also predispose endurance trained individuals to greater levels of fatigue tolerance from intracellular mechanisms. As for MCT1, MCT4 expression was also negatively correlated ($r = -0.61$, $P < 0.05$) with blood lactate after supramaximal exercise (Thomas et al. 2005).

In summary, MCTs play an important role in both cellular metabolism and pH regulation. Specifically, MCT1 and MCT4 are important in the context of high-intensity exercise and function to facilitate lactate and H^+ efflux out of the muscle cell. Therefore, both

MCT1 and MCT4 are likely to play important roles in bridging the intracellular and extracellular bicarbonate buffering systems during high-intensity exercise.

2.2.6 Buffering during high-intensity exercise

The demand on pH regulation within the muscle cell increases substantially during the transition from rest to exercise due to greater metabolic flux (Juel 2006). During brief high-intensity exercise with a significant anaerobic glycolysis component there is a concomitant increase of lactate (La^-) and H^+ ions in both working muscle and blood due to the accumulation and subsequent disassociation of lactic acid (Thomas et al. 2005, Philp, MacDonald and Watt 2005, Thomas et al. 2007). The La^- quickly combines with another ion, such as Na^+, to produce the salt (sodium) lactate (NaLa). The accumulation of lactate and/or H^+ has been implicated as a cause of muscle fatigue (Matson and Tran 1993, Sahlin, Tonkonogi, and Söderlund 1998, Pilegaard et al. 1999, Allen, Lamb, and Westerblad 2008, Debold 2012). Nevertheless, as discussed in chapter 1, the 'lactic acidosis' theory of fatigue still divides academic opinion (Brooks 2001, Robergs, Ghiasvand, and Parker 2004, Requena et al. 2005, Lamb and Stephenson 2006, Bangsbo and Juel 2006, Cairns 2006, Allen, Lamb, and Westerblad 2008).

As previously described, during high-intensity exercise various buffering mechanisms sequester H^+ and thus prevent deleterious reductions in blood and muscular pH. As exercise progresses the intracellular buffering capacity is eventually exceeded and both lactate and H^+ diffuse into the blood. Once intracellular buffering capacity is exceeded, the extracellular buffering mechanisms are stimulated (Matson and Tran 1993) of which the [HCO_3^- / H_2CO_3] system contributes ~ 86% of total extracellular buffering capacity (Adrogué and Adrogué 2001, Poupin et al. 2012). Therefore, by augmenting the bioavailability of extracellular [HCO_3^-], through ingestion of $NaHCO_3$, more H^+ can be neutralised and exercise capacity might be prolonged (Begum, Cunliffe, and Leveritt 2005, Cairns 2006, Edge et al. 2006).

Based on this premise a number of researchers have examined the effects of $NaHCO_3$ on exercise performance (Lavender and Bird 1989, McNaughton 1992a,b, McNaughton, Ford, and Newbold 1997, McNaughton, Dalton, and Palmer 1999, Cameron et al. 2010).

2.3 The effects of $NaHCO_3$ on whole body performance

Attempts to attenuate the perturbations in acid-base homeostasis that occur during exercise date back to the early part of the 20th century. Dennig et al. (1931) reported signs of performance benefit (indicated as increased ability to accumulate oxygen debt) with induced metabolic alkalosis (using [$NaHCO_3$] ingestion) but performance decrement, (indicated as decreased ability to accumulate oxygen debt) with induced metabolic acidosis (using ammonium chloride [NH_4Cl] ingestion) during 15 minutes steady state treadmill running at 9.3 $Km.h^{-1}$. Although there are a number of methodological questions about this particular study it was the start of a thread of research, which runs to the present day evaluating how metabolic "buffers" such as $NaHCO_3$ affect exercise performance. Although research examining the efficacy of $NaHCO_3$ on exercise performance dates back to the early part of the 20th century the majority of research in this area spans the last 5 decades (Jones et al. 1977, MacLaren and Morgan 1985, McNaughton 1992a,b, McNaughton, Dalton and Palmer 1999, Price, Moss and Rance 2003, Vanhatalo et al. 2010). However, despite the proposed biochemical basis for augmented H^+ buffering empirical research has delivered varied results (Matson and Tran 1993, Ball, Greenhaff and Maughan 1996, Requena et al. 2005, McNaughton, Siegler, and Midgley 2008, Price and Simons 2010, Peart, Siegler, and Vince 2012). The variable results in research evaluating the efficacy of $NaHCO_3$ on exercise performance are due, at least in part, to variations in the dosage administered, degree of metabolic alkalosis induced, the intensity, duration and nature of the exercise undertaken, participant training status and pre-experimental procedures (Matson and Tran 1993, Maughan 1999, McNaughton, Siegler, and Midgley 2008, Peart, Siegler, and Vince 2012).

The remaining content of this chapter will consider, inter alia, how the aforementioned variations in experimental approach impact on the efficacy of $NaHCO_3$ as an ergogenic aid.

2.3.1 Dosage

Administration dosage plays a significant role as to whether an ergogenic effect is observed with $NaHCO_3$ supplementation. Research by McKenzie et al. (1986) consisted of completing 6 x 60 s cycling bouts at 125% maximal oxygen uptake ($\dot{V}O_{2MAX}$) 1 hour after ingesting either placebo (PLA), 0.15 $g.kg^{-1}$ $NaHCO_3$, or 0.3 $g.kg^{-1}$ $NaHCO_3$. The final bout was continued until exhaustion and used as the performance trial. Total work done (TWD) and performance time to exhaustion (T_{LIM}) were significantly greater for both $NaHCO_3$ trials compared to PLA. However, there were no differences between the $NaHCO_3$ trials for TWD and T_{LIM} (133 kJ vs. 133 kJ and 111 s vs. 106 s for the 0.15 $g.kg^{-1}$ and 0.3 $g.kg^{-1}$ $NaHCO_3$ trials, respectively). No differences between the $NaHCO_3$ trials were observed despite significantly higher pre-exercise pH and $[HCO_3^-]$ for the 0.3 $g.kg^{-1}$ compared to the 0.15 $g.kg^{-1}$ dosage, respectively. However, when analysing the absolute changes in pH (7.42 vs. 7.40) and $[HCO_3^-]$ (27.9 vs. 25.4 $mmol.l^{-1}$) it is plausible that such changes were not sufficiently biologically different and perhaps contributed to why no difference was observed between $NaHCO_3$ trials. Subsequent research by Horswill et al. (1988) demonstrated that despite significantly elevated blood bicarbonate levels (26.8 to 30.6 $mmol.l^{-1}$) and (26.1 to 30.9 $mmol.l^{-1}$) with ingestion of 0.15 $g.kg^{-1}$ and 0.20 $g.kg^{-1}$ $NaHCO_3$, respectively, there was no significant difference in the work performed during a 2 mins cycle sprint compared to PLA or 0.10 $g.kg^{-1}$ $NaHCO_3$. Furthermore, no trend of improvement in work done with increased dose was observed. This led the authors to suggest that a specific threshold increase of pH was required and that this might be achieved ingesting dosages higher than 0.20 $g.kg^{-1}$ $NaHCO_3$ (Horswill et al. 1988).

A key study in examining the efficacy of different dosages of $NaHCO_3$ was completed by McNaughton (1992a). This study consisted of completing 1 min of sprint cycling on seven separate occasions after consuming; a PLA (calcium carbonate [$CaCO_3$], 0.5 $g.kg^{-1}$; PLA), nothing (CON) and $NaHCO_3$ (dosages of 0.1, 0.2, 0.3, 0.4 and 0.5 $g.kg^{-1}$ $NaHCO_3$). Participants completed more work in the 0.2 ($P < 0.05$), 0.3, 0.4 and 0.5 $g.kg^{-1}$ ($P < 0.005$) trials compared to CON with the 0.3 $g.kg^{-1}$ dose facilitating the highest peak power output and work done (Figure 2.2). Despite showing no further improvement in performance, the 0.4 and 0.5 $g.kg^{-1}$ doses recorded higher levels of gastrointestinal (GI) distress leading the authors to conclude that the optimal dose of $NaHCO_3$ for 60 s of sprint cycling is 0.3 $g.kg^{-1}$, a dosage that has since been adopted widely (Kozak-Collins, Burke, and Schoene 1994, Horlidge-Horvat et al. 2000, Price and Simons 2010, Cameron et al. 2010, Price and Cripps 2012).

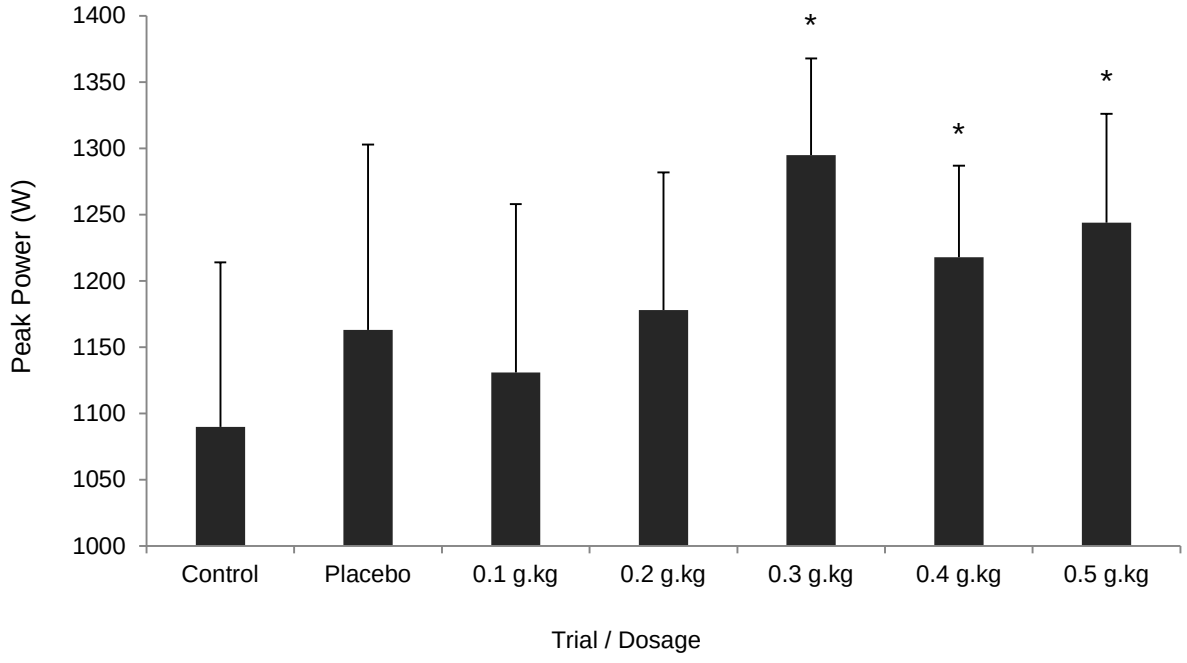

Figure 2.2 Peak power (W) during 60 s sprint cycling following control, placebo and doses of $NaHCO_3$ of 0.1 to 0.5 $g.kg^{-1}$. * $P < 0.05$ compared to CON (redrawn from McNaughton 1992a).

Despite the results from McNaughton (1992a) resulting in 0.3 $g.kg^{-1}$ becoming the 'gold standard' $NaHCO_3$ dosage, earlier research by Goldfinch, McNaughton, and Davies (1988) demonstrated that a higher dose (0.4 $g.kg^{-1}$) facilitated a significantly faster 400 m running performance (1.52 s and 1.69 s faster than CON and PLA ($CaCO_3$) respectively). This equated to a difference of ~ 10.5 and 11.5 m which was noted to be often the difference between first and last place (Goldfinch, McNaughton, and Davies 1988). However, several subjects experienced minor acute GI upsets as early as 30 mins post-exercise, and thus there appears to be a delicate balance between dosage, performance improvement and potential negative side-effects. In summary, to minimise GI discomfort (for human studies) and optimise the probability of ergogenic benefit, a dosage of 0.3 $g.kg^{-1}$ $NaHCO_3$ was adopted (McNaughton 1992a). Such a dosage typically achieves an increase of ~ 7 mM [HCO_3^-] (25 to 32mM; Price and Singh 2008, Lindh et al. 2008, Cameron et al. 2010, Siegler et al. 2010).

2.3.2 Timing of ingestion

Despite the considerable research evaluating $NaHCO_3$ as an ergogenic aid there has been little consistency in the timing of exogenous ingestion. Studies have used ingestion periods of 3 hours pre-exercise (Jones et al. 1977[1], Sutton, Jones, and Toews 1981[2], McCartney, Heigenhauser, and Jones 1983), 2.5 hours pre-exercise (Parry-Billings and MacLaren 1986, George and MacLaren 1988[3], Gaitanos et al. 1991, Tiryaki and Atterbom 1995), 2 hours pre-exercise (Wilkes, Gledhill, and Smyth 1983[4], Iwaoka et al. 1989[5], Kozak-Collins, Burke, and Schoene 1994), 1.75 hours pre-exercise (Webster et al. 1993), 1.5 hours pre-exercise (McNaughton 1992b, Linderman et al. 1992, McNaughton, Ford, and Newbold)

[1] Ingested over 3 hour period. No detailed schedule provided

[2] Ingested every 15 mins over 3 hours,

[3] Ingested in equal amounts every 30 mins over 2.5 hr period

[4] Ingested over 2 hour period

[5] Ingested 4 sets of capsules every 15 mins 2 hours pre-exercise

and 1 hour pre-exercise (Costill et al. 1984, Katz et al. 1984, McKenzie et al. 1986, Gao et al. 1988, Goldfinch, McNaughton, and Davies 1988, McNaughton 1992a, Price, Moss, and Rance 2003, Price and Simons 2010, Cameron et al. 2010).

To determine the time-course of $NaHCO_3$ supplementation, Price and Singh (2008) measured the post-ingestion blood pH and bicarbonate concentrations every 30 mins for 180 mins after ingesting a 0.3 $g.kg^{-1}$ bolus of $NaHCO_3$. The greatest increases in blood pH and blood bicarbonate concentration were achieved between 60 and 90 mins and at 60 mins, respectively, suggesting that when ingesting a bolus solution of 0.3 $g.kg^{-1}$ of $NaHCO_3$ an ingestion period of ~ 60 mins is likely to provide maximum opportunity for enhancing exercise capacity (Figure 2.3).

Figure 2.3 Blood pH and blood bicarbonate concentrations at rest and at 30 minute intervals for 3 hours after ingestion of a sodium bicarbonate solution. *Significantly different from 0 minute ($P < 0.05$); †significantly different from 60 minutes ($P < 0.05$). Reproduced in full with permission (Price and Singh 2008).

The data presented by Price and Singh (2008) supports earlier work by Renfree (2007) whose research also suggested that optimal H^+ buffering occurs between 60 and 90 mins post-ingestion of 0.3 $g.kg^{-1}$ of $NaHCO_3$ and later work by Siegler et al. (2010) who demonstrated that blood buffering capacity (pH, base excess (BE) and [HCO_3^-] measured) was significantly elevated after 60 mins when compared to 80, 100 and 120 mins after ingestion of 0.3 $g.kg^{-1}$ $NaHCO_3$. Combined, this evidence suggests that optimal ergogenic benefit may be achieved by ingestion 1 hour prior to exercise and, in part, might contribute to why some studies that have not observed ergogenic effects (McCartney et al*.* 1983, Parry-Billings and MacLaren 1986, Lambert et al*.* 1993, Kozak-Collins, Burke, and Schoene 1994, Stephens et al*.* 2002). However, it should be acknowledged that even when a 1 hour administration period is chosen, performance benefits are not automatically observed (Katz et al. 1984, Horswill et al. 1988, Vanhatalo et al*.* 2010). Moreover, even when ingestion time has not been 'optimal', performance benefits have still been observed (Jones et al. 1977, Sutton et al. 1981, Iwaoka et al. 1989, Swank and Robertson 1989, Lavender and Bird 1989, McNaughton, Ford, and Newbold 1997). Furthermore, Siegler et al. (2010) suggest that administration timing is important related to the dosage administered. This research showed that peak buffering occurred after 40 to 50 mins after ingesting 0.2 $g.kg^{-1}$ $NaHCO_3$ compared to 60 mins after ingesting 0.3 $g.kg^{-1}$ $NaHCO_3$, despite no differences in blood buffering capacity (pH, BE and [HCO_3^-]) between trials. Indeed, such observations provide further evidence related to the equivocal nature of research in this area. However, exercise performance was not measured by Siegler et al. (2010) and therefore more research is required to demonstrate if such changes translate into differences in performance. In summary, to optimise the probability of ergogenic benefit, for the human studies in this thesis an ingestion period of 60 mins was adopted (Renfree 2007, Price and Singh 2008, Siegler et al. 2010).

2.3.3 Pre-experimental procedures

Detecting changes outside of daily biological variance in exercise performance / capacity is important to nutritional supplementation research (Sewell and McGregor 2008). To minimise systematic bias, participants often perform a number of familiarisation tests before experimental data is collected (Lavender and Bird 1989, Carey and Richardson 2003, Hill et al. 2007). If enough such trials are undertaken, learning effects or other systematic changes are reduced sufficiently so that reliable performance data can be collected (Hopkins 2000). However, in the research evaluating the efficacy of $NaHCO_3$ on exercise performance / capacity there is little consistency of approach in this regard which is likely to have contributed to the inconsistent experimental results (Requena et al. 2005). For example, a number of studies incorporating T_{LIM} do not appear to have employed any familiarisation trials (Katz et al. 1984, Costill et al. 1984, McKenzie et al. 1986, Iwaoka et al. 1989, Kozak-Collins, Burke, and Schoene 1994, Price and Simons 2010) whilst others don't provide enough information to evaluate pre-experimental trial procedures (Rupp et al. 1983, MacLaren and Morgan 1985). Research using other exercise protocols is similarly inconsistent. For example, some studies do not report that participants undertook any familiarisation trials prior to experimental trials (Verbitsky et al. 1997, Price, Moss, and Rance 2003) and although McNaughton, Ford and Newbold (1997) afforded participants one control/habituation trial before experimental trials (60 s maximal work on cycle ergometer) participants were not given a warm up. Furthermore, although Mitchell et al. (1990) afforded participants two familiarisation trials, these were separated by 4 to 12 weeks. Such a time lag between trials is unlikely to have been suitable in fully familiarising participants to the experimental protocol. In contrast Lavender and Bird (1989) incorporated three full familiarisation sessions prior to experimental data collection.

Despite being the most reliable physical performance test (Hopkins, Schabort, and Hawley 2001) due to the wide range of possible exercise modalities and protocols there is a relative paucity of research evaluating the reliability of T_{LIM}. As already stated, at the time of commencing this research (Jan 2010) no research had reported the reliability of 110% T_{LIM}

using the minimum of 3 trials as recommended by Hopkins (2000). However, we acknowledge a recent publication by Saunders et al. (2012) who addressed the reliability of T_{LIM} at 110% W_{MAX}, which was published whilst our work was under peer review. Our approach in evaluating the reliability of 110% W_{MAX} T_{LIM} can be found in chapter 4 (study 1).

2.3.4 Exercise duration

Despite the biochemical basis for exogenous $NaHCO_3$ supplementation providing performance benefits, research has failed to provide consistent and positive performance effects. For example, Parry-Billings and MacLaren (1986) failed to demonstrate ergogenic benefit during 3 x repeated Wingate anaerobic test (WAnT) performance (6 mins between bouts). However, the duration of exercise (30 seconds) might have been insufficient for beneficial results. Although glycogenolytic processes are initiated almost immediately during maximal dynamic exercise (Boobis, Williams, and Wootton 1982, Spriet 1990, Smith and Hill 1991) maximum rates of glycolysis appear to occur after ~ 10 to 15 s during a WAnT test in healthy participants (Smith and Hill 1991). It should be pointed out here that Smith and Hill (1991) did not directly measure the glycolytic response. This was calculated based on a number of assumptions and derived by subtracting the ATP-PCr and aerobic contributions from the total work performed. As such, it is plausible that some small deviations from these figures might occur when directly measuring glycolytic response. Irrespective of such potential small deviations during a WAnT test in healthy non-trained individuals a significant contribution to ATP re-synthesis is likely derived from the creatine kinase (CK) reaction. This biochemical process absorbs protons and therefore acts as an initial cellular buffer by minimising falls in pH_i. Consequently, exogenously delivered HCO_3^- might have limited effect because the maximum buffering capacity is not utilised during that timeframe (Parry-Billings and MacLaren 1986). The lack of ergogenic benefit when ingesting $NaHCO_3$ prior to high-intensity exercise of less than 1 min has further support in the literature (McCartney et al. 1983, Katz et al. 1984, McNaughton 1992b) although this is not universal (Bishop et al.

2004, Douroudos et al. 2006). Interestingly, the contribution of aerobic and anaerobic energy sources during a WAnT also differs based on training status. For example, Granier et al. (1995) demonstrated that during a 30 s WAnT the contribution from aerobic and anaerobic energy sources for sprinters was ~ 25% and ~ 75%, respectively, and for endurance runners, ~ 40% and 60%, respectively. Therefore, training status and associated morphology (i.e. fibre type distribution) are likely to be important in evaluating performance outcomes (section 2.3.5).

McNaughton (1992b) demonstrated that $NaHCO_3$ ingestion had no effect on total work (TWD) and peak power (PPO) for cycling trials of 10 s and 30 s duration. However, PPO was significantly greater than CON and PLA for the trials lasting 120 s and 240 s. This equated to a ~ 8% greater PPO in the 120 s trial (both conditions) and 8.5% and 12% higher PPO in the 240 s trial for $NaHCO_3$ compared to CON and PLA, respectively (Figure 2.4). The ergogenic effects were mirrored by significantly greater blood lactate (BLa) concentrations, whose augmented transfer out of the cell is likely to account for at least part the extra work completed in $NaHCO_3$ trials (McNaughton 1992b).

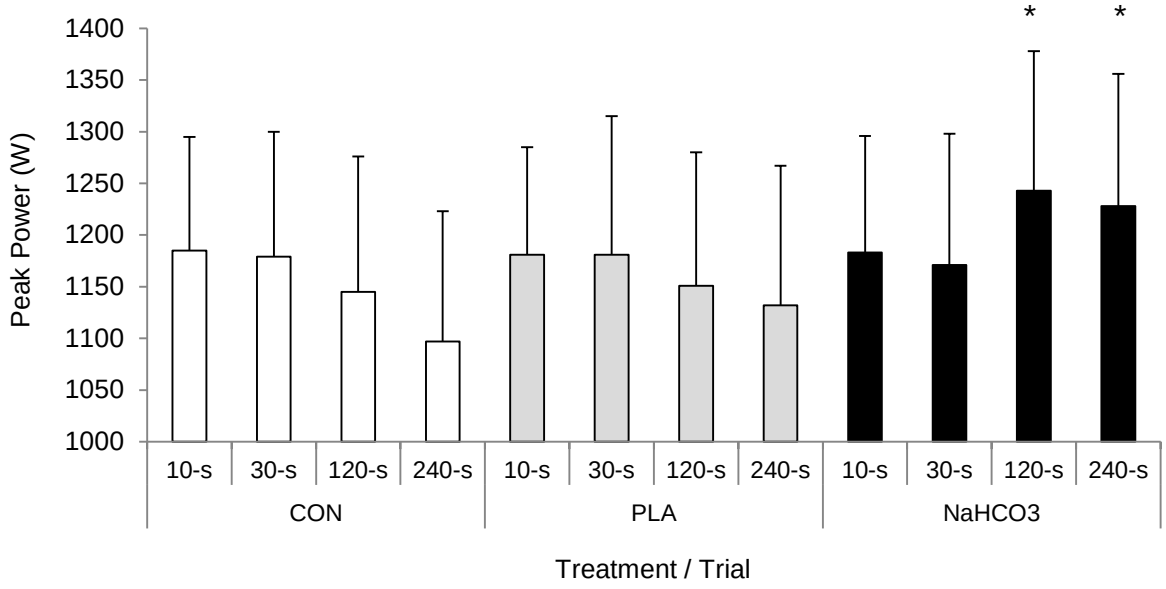

Figure 2.4 Peak power (W) during 10 s, 30 s, 120 s and 240 s high-intensity cycling following 0.3 $g.kg^{-1}$ $NaHCO_3$, 0.3 $g.kg^{-1}$ $CaCO_3$ (placebo) and nothing (CON). * $P < 0.05$ vs. CON and PLA of same duration (redrawn from McNaughton 1992b).

Previous research by Costill et al. (1984) involved participants completing four 1 min cycling bouts at 125% $\dot{V}O_{2MAX}$ (1 min rest between bouts) with a 5th until exhaustion (T_{LIM}) after consuming either 0.2 $g.kg^{-1}$ of $NaHCO_3$ or a 1 g NaCl drink. Both were dissolved in 400 ml of water and consumed 1 hour before exercise. T_{LIM} was 42% longer for alkalosis compared to PLA with times of 161 s and 114 s respectively ($P < 0.01$). Such work supports the hypothesis provided by Katz et al. (1984) who found no significant difference in T_{LIM} for induced alkalosis compared to PLA using a single cycling bout of 125% $\dot{V}O_{2MAX}$ but hypothesised that increasing exercise time through repeated bouts, presumably of similar intensity / duration, would likely result in an ergogenic effect being observed. In contrast, Bouissou et al. (1988) found a 22% increase in T_{LIM} at 125% $\dot{V}O_{2MAX}$ in trained runners undertaking cycling exercise after 0.3 $g.kg^{-1}$ $NaHCO_3$. However, Katz et al. (1984) used a dosage of 0.2 $g.kg^{-1}$ $NaHCO_3$ and did not adopt any familiarisation trials which, in addition to difference in training status, might help explain differences in exercise capacity between the results presented by Katz et al. (1984) and Bouissou et al. (1988).

Although the vast majority of research evaluating the efficacy of $NaHCO_3$ supplementation has adopted short-term high-intensity exercise protocols (up to ~ 10 mins) McNaughton, Dalton, and Palmer (1999) reported that participants performed significantly greater work (14%; $P < 0.01$) for $NaHCO_3$ compared to PLA and CON during 1 hour cycling. To optimise performance (i.e. maximum work done) during the 1 hour cycling participants had to cycle at or above the lactate threshold and therefore $NaHCO_3$ supplementation might have allowed for greater contractile performance due to augmented efflux of lactate and H^+ from working muscles (McNaughton, Dalton, and Palmer 1999). In summary, $NaHCO_3$ seems most likely to exert ergogenic benefit during high-intensity exercise of 1 to 7 mins (Linderman and Fahey 1991, Matson and Tran 1993, Linderman and Gosselink 1994) or where participants exercise at or above the lactate threshold (McNaughton, Dalton, and Palmer 1999). Indeed, Requena et al. (2005) suggested that the equivocal nature of results in this area might be due to not all exercise protocols challenging muscle buffering capacity

equally. Therefore buffer efficacy might be, at least in part, protocol specific. Interestingly, the effects of $NaHCO_3$ on exercise capacity over a range of durations within the same population have yet to be confirmed. Such evidence might help elucidate how $NaHCO_3$ affects exercise capacity (Price and Simons 2010) and confirm whether individuals can be classified as either responders or non-responders to $NaHCO_3$ (Price and Simons 2010, Saunders et al. 2011). As such, we evaluated the effects of $NaHCO_3$ on exercise capacity over a range of durations within the same population (study 2, chapter 5).

2.3.5 Training status

The wide variation in participant training status (both between and within studies) might also affect results in the area of $NaHCO_3$ research. Male participant cohorts have included trained adult cyclists (Linderman et al. 1992), runners (Wilkes, Gledhill, and Smyth, 1983, Goldfinch, McNaughton, and Davies 1988, Bird, Wiles, and Robbins 1995), wrestlers (Aschenbach et al. 2000), rugby players (Cameron et al. 2010) trained youth swimmers (Zajac et al. 2009) and moderately trained individuals (Iwaoka et al. 1989, Price, Moss, and Rance 2003, Price and Simons 2010). Female participant cohorts have included well trained adult cyclists (Kozak-Collins, Burke, and Schoene 1994) and moderately trained individuals (McNaughton, Ford, and Newbold 1997). As such it's plausible that such cohort heterogeneity, where effects might also differ between exercise modes, could contribute to the equivocal results observed in this research area.

Linderman et al. (1992) examined the effects of $NaHCO_3$ on T_{LIM} at 100% $\dot{V}O_{2MAX}$ in well trained cyclists. The authors reported no ergogenic benefit and suggested that the highly trained status of their participants may have, at least partly, negatively affected the ability of $NaHCO_3$ to demonstrate ergogenic benefit. Similarly, Brien and McKenzie (1989) suggested the lack of ergogenic benefit observed after $NaHCO_3$ ingestion in Olympic rowers might be related to a greater intracellular buffer capacity. Finally, Aschenbach et al. (2000)

speculated that the highly adapted qualitative musculature of the well trained wrestlers in their study may have contributed to why no ergogenic benefit was observed. Specifically, Aschenbach et al. (2000) suggested that their well trained participants might possess a high intracellular buffer capacity of carnosine which leaves little room for augmented extracellular buffering to be utilised. This is supported by Parkhouse et al. (1985) who demonstrated that marathon runners and untrained subjects had significantly lower levels of intracellular carnosine and overall buffering capacity than rowers or sprint-trained individuals, grouped respectively. Moreover, they also reported low/moderate but significant interrelationships between buffer capacity and carnosine levels ($r = 0.69$), buffer capacity and fast-twitch fibre composition ($r = 0.51$) and carnosine levels and fast-twitch fibre composition ($r = 0.46$). It was speculated that elevated carnosine levels and buffering capacity might be a function of high-intensity training (Parkhouse et al. 1985). Indeed, Suzuki et al. (2004) reported a 113% increase in carnosine levels after 8 weeks cycling sprint training in untrained males.

Begum, Cunliffe, and Leveritt (2005) suggest that cells have evolved different proton buffering mechanisms to defend against changes in intracellular pH. They suggest intracellular non-bicarbonate buffering is predominated by the imidazole group which exists in free histidine, histidine residues and dipeptides such as carnosine (Figure 2.5). Therefore, whilst $NaHCO_3$ may be an effective extracellular buffer, other non-bicarbonate buffering mechanisms, such as carnosine, might preclude $NaHCO_3$ from demonstrating an ergogenic effect in specific trained populations (Parkhouse et al. 1985, Brien and McKenzie 1989, Matson and Tran 1993, Aschenbach et al. 2000, Derave et al. 2010).

Figure 2.5 Metabolism of carnosine (Begum, Cunliffe, and Leveritt 2005).

The relationship between training status and observation of ergogenic benefit with $NaHCO_3$ ingestion is not definitive. Ergogenic benefit with $NaHCO_3$ supplementation has been reported in highly trained runners (Goldfinch, McNaughton, and Davies 1988, Bird, Wiles, and Robbins 1995) and cyclists (Driller et al. 2012ab). Furthermore, McNaughton, Dalton, and Palmer (1999) reported that well trained cyclists performed significantly greater work (14%) for $NaHCO_3$ compared to PLA and CON during 1 hour cycling. Paradoxically, a study using a protocol of similar duration to that adopted by McNaughton, Dalton, and Palmer (1999) found that time to complete a fixed amount of work (~ 470 kJ, ~ 30 mins) after an initial 30 mins at ~ 77% $\dot{V}O_{2PEAK}$ was unaffected by $NaHCO_3$ supplementation (Stephens et al. 2002). However, small, yet important, differences in experimental methodology (ingestion period, exercise protocol) might have played a role in the differing results for Stephens et al. (2002). Finally, it is possible that despite selection of potentially inappropriate participants (i.e. endurance trained athletes completing an all-out maximal type protocol) ergogenic effects might still be observed (Van Montfoort et al. 2004). The mechanisms are somewhat unclear but could be related to the specific interval training undertaken by these athletes, distance runners, which included brief high-intensity efforts (Van Montfoort et al.

2004). Such training is likely to provide an appropriate experimental (i.e. familiarity with exercise intensity) and physiological (i.e. sufficient type II muscle fibre development) environment to optimise the chance of ergogenic benefit of $NaHCO_3$. Moreover, it is plausible that a lack of volume of high-intensity training, such as in sprinters, in these individuals did not augment intracellular buffering sufficiently to negate the utilisation of augmented extracellular buffering from $NaHCO_3$. However, this is largely speculative and warrants further investigation.

Although a number of studies have examined the effects of $NaHCO_3$ ingestion immediately *prior* to high-intensity training on a variety of physiological and performance parameters (Edge, Bishop, and Goodman 2006, Thomas et al. 2007, Bishop et al. 2010, Driller et al. 2012c), no research has evaluated the efficacy of $NaHCO_3$ supplementation after a change in training status (i.e. before and after (not during) a period of training). As an individual's training status might affect responses to $NaHCO_3$ during exercise (Linderman et al. 1992, Aschenbach et al. 2000) and because untrained individuals are more likely to observe ergogenic benefit than trained individuals (Peart, Siegler, and Vince 2012) we evaluated the efficacy of $NaHCO_3$ on exercise capacity in non-cycling trained males before and after 6 weeks high-intensity cycling training (study 4, chapter 7). Similar research has demonstrated training induced physiological changes (i.e. augmented levels of intracellular carnosine) that might impact the efficacy of $NaHCO_3$ in this population (Suzuki et al. 2004).

2.3.6 Exercise Mode

The efficacy of $NaHCO_3$ as an ergogenic aid has been examined in numerous exercise modes including running (Kindermann, Keul, and Huber 1977, MacLaren and Mellor 1985, Potteiger et al. 1996, Price and Simons 2010), leg press capacity (Webster et al. 1993, Portington et al. 1998), isokinetic knee flexion and extension (Coombes and McNaughton 1993), swimming (Pierce et al. 1993, Lindh et al. 2008, Pruscino et al. 2008),

2000 m rowing performance (Nielsen et al. 2002, Carr, Gore, Dawson 2011, Carr et al. 2012, Kupcis et al. 2012), water polo (Tan et al. 2010), skilled tennis performance (Wu et al. 2010), bench press performance (Materko, Santos, and Novaes 2008), forearm exercise (Raymer et al. 2004), judo (Artioli et al. 2006) and boxing performance (Siegler and Hirscher 2010). However, despite such a wide variety of exercise modes the majority of research evaluating the effects of $NaHCO_3$ on exercise performance has used cycling as the exercise mode (Matson and Tran 1993). As such the main focus of this section will be to review the effects of $NaHCO_3$ on cycling performance. Moreover, due to the amount of data related to $NaHCO_3$ and cycling performance and thus the potential for evaluation against new research, cycling was the exercise mode chosen for all whole body studies in this thesis (chapters 4, 5 and 7).

2.3.6.i Cycling

Research examining the efficacy of $NaHCO_3$ on cycling performance / capacity extends over at least 5 decades (Table 2.1). Jones et al. (1977) reported that T_{LIM} at 95% $\dot{V}O_{2MAX}$ was 62% and 174% greater for $NaHCO_3$ compared to CON ($CaCO_3$) and metabolic acidosis (ACD: NH_4Cl), respectively, following 20 mins of exercise at 33% and 66% $\dot{V}O_{2MAX}$. Similarly Sutton et al. (1981) and Rupp et al. (1983) reported large ergogenic benefits of 19% and 34% compared to CON trials ($CaCO_3$ and lactose, respectively). In contrast Katz et al. (1984) found no difference between treatments for T_{LIM} cycling at 125% $\dot{V}O_{2MAX}$ although the authors subsequently hypothesised that adopting repeated bouts, presumably at the same or similar intensity with short recovery periods, would likely result in an ergogenic effect being observed. Indeed, MacLaren and Morgan (1985) found that T_{LIM} cycling at 100% $\dot{V}O_{2MAX}$ was 14% greater than PLA. In contrast, Bouissou et al. (1988) found a 22% increase in T_{LIM} for trained runners undertaking cycling at 125% $\dot{V}O_{2MAX}$ after ingesting 0.3 $g.kg^{-1}$ $NaHCO_3$. However, differences in training status, and the fact that participants in the Katz et al. (1984) study ingested less $NaHCO_3$ (0.2 $g.kg^{-1}$) might have contributed to the differences in results between studies. A number of studies have also evaluated $NaHCO_3$ on repeated

sprint cycling. Costill et al. (1984) and McKenzie et al. (1986) demonstrated that T_{LIM} was 42% and 45% longer for $NaHCO_3$ compared to PLA in the final 1 min bout, of 5 and 6 bouts respectively, with 1 min rest between bouts, at 125% $\dot{V}O_{2MAX}$.

A key study on repeated sprint cycling performance was undertaken by Lavender and Bird (1989). Twenty-three participants completed 10 x 10 s maximal sprints with 50 seconds rest between each sprint after either $NaHCO_3$ (ALK) or NaCl (PLA) at WAnT load (i.e. 7.5% body mass). These experimental trials were repeated three times for each treatment following three familiarisation trials. A further two trials were completed with an inert substance (CON; blackcurrant juice) to evaluate any possible ergogenic effects of the original PLA (NaCl). Mean power output (MPO) and peak power output (PPO) was significantly greater for ALK compared to PLA for 8/10 sprints (incl sprints 5-10) and sprints 2 and 10, respectively. Interestingly, PLA also showed ergogenic benefit compared to CON in both MPO and PPO for sprints 7, 10 and 6, 7, 8, respectively (Lavender and Bird 1989). In contrast, Matsuura et al. (2007) reported no differences in MPO and PPO between ALK ($NaHCO_3$) and PLA ($CaCO_3$) after 10 x 10 s cycle sprints interspersed with either 30 s or 360 s recovery. However, it's plausible that the different work-to-rest ratios and the subsequent physiological responses contributed to the differences in results. Bishop et al. (2004) evaluated the effects of $NaHCO_3$ (ALK) on 5 x 6 s all-out sprints every 30 s. Work done and PO were significantly greater during sprints 3, 4, and 5 for ALK compared to CON. In contrast Kozak-Collins, Burke, and Schoene (1994) reported no difference in the number of 1 min intervals completed at 95% $\dot{V}O_{2MAX}$ (followed by 1 min recovery at 60 W) between ALK (10 ± 0.9) and an equimolar dose of NaCl (PLA; 8.4 ± 0.9).

Price, Moss, and Rance (2003) examined the effect of $NaHCO_3$ using an exercise protocol resembling the intermittent profile of sports such as hockey or rugby. Participants completed repeated 3 minute blocks for 30 mins consisting of 90 s at 40% $\dot{V}O_{2MAX}$, 60 s at 60% $\dot{V}O_{2MAX}$, 14 s maximal efforts followed by 16 s active rest after ingestion of either

$NaHCO_3$ (ALK) or NaCl (PLA). The authors reported that ALK enabled sprint performance to be maintained similar to initial maximal sprint efforts whereas sprint performance declined for PLA. In a longer protocol, consisting of 2 x 36 mins periods of intermittent exercise (IST) Bishop and Claudius (2005) reported no significant performance differences (total work) between treatments in either period of the IST. However, 7 out of 18 of the second half sprints produced significantly more work for $NaHCO_3$ compared to PLA.

The efficacy of $NaHCO_3$ on more traditional endurance cycling capacity has also been reported. Stephens et al. (2002) reported no difference in T_{LIM} (~ 80% $\dot{V}O_{2PEAK}$: aim to complete set amount of work) between ALK and $CaCO_3$ (PLA) after an initial 30 mins at ~ 77% $\dot{V}O_{2PEAK}$, in trained cyclists. Similarly, Mitchell et al. (1990) reported no difference in T_{LIM} at ~ 80 $\dot{V}O_{2MAX}$ between ALK and PLA (intravenously administered $NaHCO_3$ and NaCl, respectively) although both were significantly greater than CON (no infusion). This suggests that an exercise intensity ≥ 80 $\dot{V}O_{2MAX}$ might be required to observe ergogenic benefit with ALK. In contrast, McNaughton, Dalton, and Palmer (1999) reported that MPO was ~ 14% greater for ALK compared to PLA and CON for trained cyclists (265 W (951 kJ) compared to 233 W (839 kJ) and 232 W (836 kJ) respectively) during 60 mins maximal work.

Table 2.1 Summary of research that has evaluated the ergogenic effects of $NaHCO_3$ on cycling capacity and/or performance.

Author(s)	$NaHCO_3$ Dosage ($g.kg^{-1}$ body mass)	Protocol	n	Results	Ergogenic Benefit
Jones et al. (1977)	0.3 $g.kg.^{-1}$	20 mins each at 33% and 66% $\dot{V}O_{2MAX}$ and then 95% $\dot{V}O_{2MAX}$ until fatigue after: (1) $CaCO_3$ (CON), (2) NH_4Cl (ACD) and (3) $NaHCO_3$ (ALK)	5 males	T_{LIM} @ 95% $\dot{V}O_{2MAX}$ were 438 +/- 120 s, (ALK), 160 +/- 22 s (ACD) and 270 +/- 13 s (CON). This equates to 62% and 174% greater T_{LIM} for ALK than CON and ACD respectively	Yes
Sutton et al. (1981)	0.3 $g.kg.^{-1}$	20 mins each at 33% and 66% $\dot{V}O_{2MAX}$ and then 95% $\dot{V}O_{2MAX}$ until fatigue after: (1) $CaCO_3$ (CON), (2) NH_4Cl (ACD) and (3) $NaHCO_3$ (ALK)	5 males	T_{LIM} @ 95% $\dot{V}O_{2MAX}$ was significantly greater for ALK compared to ACD and greater for ALK compared to CON and CON compared to ACD (both n/s) with times of 5.44 +/- 1.05 min (ALK), 3.13 +/- 0.97 min (ACD) and 4.56 +/- 1.31 min (CON). This equates to 73% and 19% greater T_{LIM} for ALK than ACD and CON respectively	Yes
Rupp et al. (1983)	0.3 $g.kg.^{-1}$	20 mins at 66% $\dot{V}O_{2MAX}$, 95% until fatigue under after:(1) $NaHCO_3$ (ALK) (2) Lactose (PLA)	4 males	T_{LIM} was 34% higher with ALK	Yes
Inbar et al. (1983)	Inconsistent data reported	30 s WAnT test against 4.41 J per pedal revolution per kg body weight after: (1) $NaHCO_3$ (ALK) and (2) NaCl (PLA)	13 males (active not trained)	Significantly higher mean power outputs (1.3 %) were observed with $NaHCO_3$ ingestion compared to PLA. This was observed in 11 / 13 participants	Yes
McCartney et al. (1983)	0.3 $g.kg.^{-1}$	30 s Maximal effort at 100 $rev.min^{-1}$ under 4 conditions: (1) $CaCO_3$ (PLA), (2) NH_4Cl (ACD), (3) $NaHCO_3$ (ALK), (4) Respiratory Acidosis (5% CO_2 humidified air inspired)	6 males (regularly active)	Maximal PPO, MPO and TWD were lower for ACD but not significantly different to ALK	No
Kowalchuk, Heigenhauser, and Jones (1984)	0.3 $g.kg.^{-1}$	Continuous incremental cycling test (+ 0.2 $kg.min^{-1}$) after: (1) $CaCO_3$ (PLA), (2) NH_4Cl (ACD), and (3) $NaHCO_3$ (ALK)	6 healthy males	T_{LIM} was 7% and 8% greater for ALK and CON compared to CON, respectively. No difference was observed between ALK and CON	No

Author(s)	$NaHCO_3$ Dosage ($g.kg^{-1}$ body mass)	Protocol	n	Results	Ergogenic Benefit
Costill et al. (1984)	0.2 $g.kg^{-1}$	5 x 1 min bouts at 125% $\dot{V}O_{2MAX}$ (1 min rest), 5th until fatigue after: (1) $NaHCO_3$ (ALK) and (2) PLA (NaCl)	10 males, 1 female	T_{LIM} was 42% higher for the ALK trial compared to PLA with times of 161 s and 114 s respectively	Yes
Katz et al. (1984)	0.2 $g.kg^{-1}$ (per 6ml solution)	T_{LIM} at W 125% $\dot{V}O_{2MAX}$ after: (1) $NaHCO_3$ (ALK) and (2) NaCl (PLA)	8 males	T_{LIM} was not significantly different between trials	No
MacLaren & Morgan (1985)	0.25 $g.kg^{-1}$	T_{LIM} at 100% $\dot{V}O_{2MAX}$ after $NaHCO_3$ (ALK) or placebo (PLA)	7 males	T_{LIM} was significantly longer for $NaHCO_3$ (14%) compared to PLA	Yes
McKenzie et al. (1986)	0.15 and 0.3 $g.kg^{-1}$	6 x 1 min bouts at 125% $\dot{V}O_{2MAX}$ (1 min rest), 6th until fatigue under two conditions: under 3 conditions: (1) Low $NaHCO_3$ (LOW), (2) High $NaHCO_3$ (HIGH) and (3) Placebo (PLA)	6 males	TWD was significantly higher for LOW and HIGH compared to PLA but there were no differences between LOW and HIGH trials. T_{LIM} was also significantly higher (~ 45%) for ALK compared to PLA	Yes
Parry-Billings & MacLaren (1986)	0.3 $g.kg.^{-1}$	3 x 30 s WAnT (6 mins recovery) under 4 conditions: (1) PLA (NaCl), (2) ALK1 ($NaHCO_3$), (3) ALK2 (NaCit), (4)COMB ($NaHCO_3$ + NaCit)	6 males (active)	Mean and peak power were significantly reduced by successive WAnT tests but no differences between treatments	No
Bouissou et al. (1988)	0.3 $g.kg.^{-1}$	T_{LIM} at 125% $\dot{V}O_{2MAX}$ after: (1) $CaCO_3$ (PLA) and (2) $NaHCO_3$ (ALK)	Six healthy males (runners)	T_{LIM} was 22% greater for ALK compared to PLA	Yes
Horswill et al. (1988)	0.10, 0.15, and 0.20 $g.kg^{-1}$	4 x 2 mins sprints under 4 conditions: (1) PLA, (2) 0.10 $g.kg^{-1}$ (LOW), (3) 0.15 g.kg (MED) and 0.20 $g.kg^{-1}$ (HIGH) $NaHCO_3$	9 males (endurance trained)	Work performed during 2 mins cycle sprint was not significantly different between ALL trials despite elevated HCO_3 levels post ingestion for MED and HIGH	No
Iwaoka et al. (1989)	0.2 $g.kg^{-1}$	Cycling at 40% $\dot{V}O_{2MAX}$ for 40 mins, then 15 mins at 12% above RCT (respiratory compensation threshold) and then T_{LIM} at 95% $\dot{V}O_{2MAX}$ after (1) $NaHCO_3$ (ALK) (2) Starch (PLA)	6 males	(1) T_{LIM} for ALK was significantly greater (49%) than PLA (2.98 min vs. 2.00 min; $P < 0.05$)	Yes

Author(s)	$NaHCO_3$ Dosage ($g.kg^{-1}$ body mass)	Protocol	n	Results	Ergogenic Benefit
Lavender and Bird (1989)	0.3 $g.kg^{-1}$ (200ml 1hr and 200ml 2hrs pre)	10 x 10 s maximal sprints with 50 seconds rest between each one (against pre-calculated load from WAnT test) under 3 conditions: (1) 3 x $NaHCO_3$, (2) 3 x PLA (NaCl), (3) 2 x CON	8 males and 15 females (n = 12 for CON)	MPO was significantly greater for ALK than PLA for 8/10 sprints (incl sprints 5-10) and PPO was greater in sprints 2 and 10. PLA showed ergogenic effect vs. CON in both MPO and PPO for sprints 7, 10 and 6, 7, 8 respectively	Yes
Cho et al. (1990)	0.3 $g.kg^{-1}$	T_{LIM} during 1-km and 3-km time trial and $\dot{V}O_{2MAX}$ assessment under after: (1) $NaHCO_3$ (ALK) and (2) $CaCO_3$ (PLA)	6 competitive cyclists	T_{LIM} was not different for 1-km but was 1.5% faster in 3-km race for ALK compared to PLA. $\dot{V}O_{2MAX}$ was 5.5% greater for ALK compared to PLA	Yes
Mitchell et al. (1990)	~ 20 g in total (1.3% of 1.5 L solution)	~ 80 $\dot{V}O_{2MAX}$ to exhaustion under 3 conditions: (1) $NaHCO_3$ (ALK) (2) NaCl (PLA; 13.5 g in 1.5) - both intravenously administered - and (3) no infusion (CON)	8 males	T_{LIM} was significantly greater (~ 68%) for both ALK and PLA compared to CON with TLIM of 31.9 ± 5.8 min, 31.8 ± 4.1 min and 19.0 ± 2.9 min respectively but no differences observed between ALK and PLA	No
McNaughton et al. (1991)	0.4 g $.kg^{-1}$	60 s maximal work under three conditions: (1) ALK ($NaHCO_3$), (2) PLA ($CaCO_3$) and (3) control (CON)	8 males (well trained)	During ALK 7% more work was completed compared to both PLA and CON	Yes
Housh et al. (1991)	0.3 $g.kg.^{-1}$	Discontinuous and continuous cycling at fatigue threshold after: (1) $NaHCO_3$ (ALK) and (2) NH_4Cl (ACD)	18 males	There was no difference in working capacity (PO) at the fatigue threshold in either condition	No
Linderman et al. (1992)	0.2 $g.kg^{-1}$	100% $\dot{V}O_{2MAX}$ (70 $rev.min^{-1}$) under 4 conditions: (1), PLA, (2) P/B, (3) PAK and (4) ALK ($NaHCO_3$)	8 males (well trained)	There was no significant difference between all 4 trials in the ability to maintain Power-max.	No
McNaughton (1992a)	0.3 $g.kg^{-1}$	60 s maximal work under 7 conditions (1) PLA ($CaCO_3$), (2) CON, (3)-(7) $NaHCO_3$ with 0.1 $g.kg^{-1}$ to 0.5 $g.kg^{-1}$ (with 0.1 $g.kg^{-1}$ increases)	9 active males	Participants completed more work in the 0.2 ($P < 0.05$), 0.3, 0.4 and 0.5 g.kg ($P < 0.005$) trials compared to CON and PLA with a 0.3 $g.kg^{-1}$ dose providing the optimal ergogenic effect	Yes
McNaughton (1992b)	0.3 $g.kg^{-1}$	Max work in 10, 30, 120 and 240 s under 3 conditions: (1) ALK ($NaHCO_3$) (2) PLA ($CaCO_3$) and (3) CON	4 * 8 males (aerobically active)	ALK had no effect on TWD and PPO for 10 and 30 s trials but was , significantly higher in the 120 and 240 s trials	Yes

Author(s)	$NaHCO_3$ Dosage ($g.kg^{-1}$ body mass)	Protocol	n	Results	Ergogenic Benefit
Lambert et al (1993)	0.3 $g.kg^{-1}$	5 mins cycling at 70, 80 and 90% $\dot{V}O_{2MAX}$, with 5 mins rest periods and then 100% $\dot{V}O_{2MAX}$ to exhaustion after: (1) $NaHCO_3$ (ALK) and (2) $CaCO_3$ (PLA)	6 males	T_{LIM} was NOT significantly different between conditions with times of 173 s and 184s for ALK and PLA respectively	No
Kozak-Collins, Burke, and Schoene 1994	0.3 $g.kg^{-1}$	1 min intervals at 95% $\dot{V}O_{2MAX}$, 1 min recovery (60W) till exhaustion after: (1) $NaHCO_3$ (ALK) and (2) NaCl (PLA; equimolar dose)	7 females (competitive cyclists)	No difference was found in the number of intervals completed between two trials	No
Verbitsky et al. (1997)	0.4 $g.kg^{-1}$	2 mins fatiguing FES, 3 mins cycling, 2 mins post-exercise FES and then FES during 40 mins recovery period (only for 2 and 3) under 3 conditions: (1) 100% $\dot{V}O_{2MAX}$ (CON1), (2) 117% $\dot{V}O_{2MAX}$ (CON2) and (3) 117% $\dot{V}O_{2MAX}$ + $NaHCO_3$ ingestion (ALK)	6 males	Peak and residual torque was significantly higher post 3 mins cycling for ALK compared to CON1 and CON2. Post-load torque was also higher than pre-load torque for ALK but lower for CON1 and CON2. During recovery peak torque was higher in ALK than CON2	Yes
McNaughton et al. (1997)	0.3 $g.kg^{-1}$	1 x 60 s maximal bout under 3 conditions: (1) CON, (2) PLA (NaCl) and (3) ALK ($NaHCO_3$). PLA was equimolar to experimental treatment)	10 females (moderately active but non cycling trained)	TWD and PPO were significantly higher for $NaHCO_3$ than CON and PLA with $NaHCO_3$	Yes
McNaughton et al. (1999)	0.5 $g.kg^{-1}$ for 5 days (chronic) taken in 4 equal amounts	60 s HIE on cycle ergometer under 3 conditions: (1) Pre-ingestion (Pre), (2) experimental (5 days supp with $NaHCO_3$; ALK) and (3) One month post (CON) trials.	8 males (7 metabolite data)	TWD and PPO significantly higher for ALK compared to CON and Pre	Yes
McNaughton, Dalton and Palmer (1999)	0.3 $g.kg^{-1}$	60 mins cycle - maximal work completed under 3 conditions: (1) ALK ($NaHCO_3$) and (2) PLA (NaCl; equimolar dose) and (3) CON	10 males (highly trained)	Average power was significantly greater for ALK (~ 14%) than PLA and CON (265 W (951 kJ) compared to 233 W (839 kJ) and 232 W (836 kJ) respectively).	Yes
McNaughton and Thompson (2001)	0.5 $g.kg^{-1}$	3 x 90 s maximal work (1 bout on 3 separate days) after: (1) acute ingestion (bout 1 only) of $NaHCO_3$ (AI) or(2) 5-days chronic ingestion of $NaHCO_3$ (CI)	8 males	Significantly more work was completed during AI and CI compared to CON during test 1. However, more work was also completed in tests 2 and 3 compared to CON for CI but not AI.	Yes

Author(s)	$NaHCO_3$ Dosage ($g.kg^{-1}$ body mass)	Protocol	n	Results	Ergogenic Benefit
Stephens et al. (2002)	0.3 $g.kg^{-1}$	30 mins @ ~ 77% $\dot{V}O_{2PEAK}$ and then ~ 30 mins @ ~ 80% $\dot{V}O_{2PEAK}$ (aim to complete set amount of work) after: (1) $NaHCO_3$ (ALK) and (2) $CaCO_3$ (PLA)	7 trained males	Performance times to complete the set amount of work (part 2) were not significantly different between treatments	No
Marx et al. (2003)	0.3 $g.kg^{-1}$	90 s maximal work after: (1) $NaHCO_3$ (ALK) and (2) NaCl (PLA). Both solutions were mixed with dextrose	10 males	There were no differences in mean or peak power, or total work between ALK and CON	No
Price, Moss, and Rance (2003)	0.3 $g.kg^{-1}$	Repeated 3 mins blocks (for 30 mins consisting of: 90s at 40% $\dot{V}O_{2MAX}$ 60s at 60% $\dot{V}O_{2MAX}$, 14s maximal effort, 16s active rest after: (1) $NaHCO_3$ (ALK) and (2) NaCl (PLA)	8 males (moderately trained)	$NaHCO_3$ ingestion enabled sprint performance to be maintained similar to initial maximal sprint during 30 mins of high-intensity intermittent exercise than compared to PLA	Yes
Bishop et al. (2004)	0.3 $g.kg^{-1}$	5 x 6 s all-out sprints every 30 s after:(1) $NaHCO_3$ (ALK), (2) NaCl - equimolar to ALK (PLA)	10 females (moderately active but non cycling trained)	Significantly more work was completed after ingesting the $NaHCO_3$ compared to CON with work and power output also significantly higher during sprints 3, 4, and 5	Yes
Bishop and Claudius (2005)	2 x 0.2 $g.kg^{-1}$	2 x 36 mins halves of intermittent exercise after: (1) $NaHCO_3$ (ALK) and (2) equimolar dose of NaCl (PLA)	7 intermittent trained females	No significant differences (total work) between treatments in either half of the IST. However, 7 / 18 of the second half sprints produced significantly more work for $NaHCO_3$ compared to PLA	Yes
Douroudos et al. (2006)	0.3 or 0.5 $g.kg^{-1}$	MPO was measured from WAnT before and after 5-days supplementation with: (1) fruit juice (PLA), (2) 0.3 $g.kg^{-1}$ $NaHCO_3$ (MED), or 0.5 $g.kg^{-1}$ $NaHCO_3$ (HIGH).	24 males	MPO was significantly greater for HIGH, but not MED or PLA after 5-days supplementation	Yes
Matsuura et al. (2007)	0.3 $g.kg^{-1}$	10 x 10 s cycle sprints interspersed with either 30 s or 360 s recovery after: (1) $NaHCO_3$ (ALK) or (2) $CaCO_3$ (PLA)	8 healthy males	No differences in MPO and PPO between ALK and PLA. Additionally there were no differences in SEMG activity between ALK and PLA	No
Zabala et al. (2008)	0.3 $g.kg^{-1}$	3 x 30 s WAnT (30 mins recovery) after: (1) $NaHCO_3$ (ALK) or (2) NaCl (PLA)	9 elite BMX riders	There were no differences in MPO, PPO, time to PPO or RPE between ALK and PLA	No

Author(s)	$NaHCO_3$ Dosage ($g.kg^{-1}$ body mass)	Protocol	n	Results	Ergogenic Benefit
Siegler et al. (2008)	0.3 $g.kg^{-1}$	T_{LIM} at 120% $\dot{V}O_{2MAX}$after: (1) $NaHCO_3$ (ALK) and (2) $CaCO_3$ (PLA). Recovery was monitored after each trial in passive/active forms (total of 4 trials)	9 males	There was no difference in T_{LIM} between ALK and PLA	No
Vanhatalo et al. (2010)	0.3 $g.kg^{-1}$	3 mins all out sprints against fixed resistance after:(1) $NaHCO_3$ (ALK) and (2) NaCl (PLA)	8 males (active)	Despite statistically significant increases in both pH and bicarbonate between treatments no performance differences found in total work done or critical power for $NaHCO_3$ and PLA respectively	No
Zinner et al. (2011)	0.3 $g.kg^{-1}$	4 x 30 sec maximal sprints interspersed by 5 mins passive after: (1) $NaHCO_3$ (ALK) and (2) $CaCO_3$ (PLA)	11 well trained males	MPO was significantly greater for ALK during sprints 3 and 4. There were no differences in PPO although the effect size for sprint 4 (0.37) suggests a possible PPO effect in the latter stages of repeated sprinting exercise with ALK	Yes
Saunders et al. (2011)	0.3 $g.kg^{-1}$	T_{LIM} at 110% W_{PEAK} after: (1) $NaHCO_3$ (ALK) and (2) Maltodextrin (PLA) to assess total work done (TWD)	21 males	No difference in TWD between ALK and PLA for group data However, when participants with GI distress were removed from the analysis (n=4), TWD was ~ 5% greater for ALK compared to PLA	Yes
Wahl et al. (2011)	0.3 $g.kg^{-1}$	3 x 30 s WAnT (15 mins recovery) after (1) $NaHCO_3$ (ALK) or (2) NaCl (PLA)	11 males	MPO was significantly greater for bouts 3 and 4 for ALK compared to PLA. Also, the fatigue index was significantly lower in bout 4 for ALK compared to PLA	Yes
Zabala et al. (2011)	0.3 $g.kg^{-1}$	4 x 30 s all-out efforts interspersed with 5 mins passive recovery and 60 mins at 50% PPO after: (1) $NaHCO_3$ (ALK) or (2) $CaCO_3$ (PLA)	10 elite BMX riders	There were no differences in MPO, PPO, time to PPO, CMJ post or RPE between ALK and PLA	No
Driller et al. (2012a)	0.3 $g.kg^{-1}$	2 mins maximal test under 3 conditions: (1) ALK ($NaHCO_3$), (2) PLA (NaCl) and CON (Maltodextrin)	8 well trained males	MPO was significantly greater in ALK compared to PLA (2.1%) and CON (3.2%). PPO was also significantly greater in ALK compared to PLA (5.8%) and CON (9.6%)	Yes
Driller et al. (2012b)	0.3 $g.kg.^{-1}$ or 0.4 $g.kg.^{-1}$ per day for 3 days (4 equal doses)	4 mins maximal test under 3 conditions: (1) Acute ALK ($NaHCO_3$), (2) Chronic ALK ($NaHCO_3$) and (3) PLA (Cellulose)	8 well trained males	MPO was 3.2% and 2.2% greater compared to PLA for Acute ALK and Chronic ALK, respectively. No differences for MPO between ALK conditions, although trend for Acute ALK being most beneficial	Yes

Overall, 62% (28 out of 45) of studies examining cycling performance reported an ergogenic benefit with exogenous $NaHCO_3$ supplementation during cycling (Table 2.1). However, when 'optimal' research characteristics such as (1) dosage (0.3 $g.kg^{-1}$; McNaughton 1992a), (2) pre-exercise ingestion time (60-90 mins; Renfree 2007, Price and Singh 2008) and (3) exercise duration (~ 1 to 7 mins of high intensity exercise (Linderman and Fahey 1991, Matson and Tran 1993, Linderman and Gosselink 1994) or where exercise intensity is at or close to the lactate threshold (McNaughton, Dalton, and Palmer 1999)) are considered, this increases to 78% (7 out of 9 studies). A further twenty two studies met two of the three optimal characteristics outlined with performance benefit dropping to 68% (15 out of 22 studies) and when only one characteristic was met this dropped to 33% (4 out of 12 studies). In summary, there appears to be a relationship between the chances of observing a performance benefit in a cycling protocol and the number of 'optimal' research characteristics already outlined having been met. However, it must be acknowledged that whilst dosage, ingestion time and exercise duration are key components of experimental design, there are many other factors that contribute to the results examining the effects of $NaHCO_3$ on exercise performance. Indeed, ergogenic benefit was still observed by Inbar et al. (1983) and Douroudos et al. (2006) when none of these experimental characteristics were met.

2.3.6.ii Running

Research evaluating the efficacy of $NaHCO_3$ on running performance dates back over 80 years. Dennig et al. (1931) reported signs of performance benefit (increased oxygen debt) with $NaHCO_3$ ingestion during 15 mins steady state treadmill running. However, although less in number, similarly to cycling the vast body of research in this area spans the last 5 decades (Kindermann, Keul, and Huber 1977, Wilkes, Gledhill, and Smyth 1983, Bird, Wiles, and Robbins 1995, Van Montfoort et al. 2004, Price and Simons 2010). Kindermann, Keul, and Huber (1977) found that $NaHCO_3$ infusion had no impact on 400 m running time

although there are a number of methodological concerns (dosage, infusion timings) with this study. In contrast Goldfinch, McNaughton, and Davies (1988) demonstrated that 0.4 $g.kg^{-1}$ $NaHCO_3$ facilitated significantly faster 400 m running performance compared to CON and PLA, the differences in time equating to the difference between first and last place in a race (Goldfinch, McNaughton, and Davies 1988). Similarly, Wilkes, Gledhill, and Smyth (1983) reported that 800 m time was faster after ingesting 0.3 $g.kg^{-1}$ $NaHCO_3$ compared to CON (2.9 s) and PLA (2.2 s), these differences also equivalent to the difference between first and last place. Finally, Bird, Wiles, and Robbins (1995) reported that 1500 m performance time was 2.9 s faster after ingesting 0.3 $g.kg^{-1}$ $NaHCO_3$ compared to PLA and 4.1 s faster compared to CON. In contrast Tiryaki and Atterbom (1995) reported no differences in 600 m time after $NaHCO_3$ ingestion compared to PLA or sodium citrate (NaCit) ingestion. However, the authors reported a significant training effect between pre and post baseline tests which might have confounded results.

Other studies have evaluated the effects of $NaHCO_3$ on running capacity (i.e. T_{LIM}) as opposed to time to complete a set distance (i.e. 400 m or 800 m). George and MacLaren (1988) reported that after ingestion of 0.2 $g.kg^{-1}$ $NaHCO_3$ endurance running at a velocity corresponding to 4.0 $mmol.l^{-1}$ blood lactate was 17% greater than PLA (NaCl) and 44% greater than metabolic acidosis (ACD; NH_4Cl). Potteiger et al. (1996) found that ingestion of 0.3 $g.kg^{-1}$ $NaHCO_3$ resulted in 29% and 66% increases in exercise capacity compared to PLA and NaCit trials, respectively, when running at 110% of the lactate threshold immediately after 30 mins running at the lactate threshold. Similarly, Van Montfoort et al. (2004) reported that 0.3 $g.kg^{-1}$ $NaHCO_3$ improved T_{LIM} against sodium lactate (2.6%), NaCit (5.2%) and NaCl (6.4%) during high-intensity treadmill running designed to elicit volitional exhaustion in ~ 1 to 2 mins with a ~ 2 % incline. Finally, Price and Simons (2010) examined the effects of $NaHCO_3$ on T_{LIM} at 120% v-$\dot{V}O_{2MAX}$ after intermittent high-intensity running akin to that adopted by individuals undertaking interval-type training. $NaHCO_3$ had no affect on T_{LIM} compared to PLA (NaCl) at a group level although there was significant variation in capacity.

This led the authors to suggest an individualised approach should be adopted for $NaHCO_3$ ingestion.

2.3.6.iii Swimming

Gao et al. (1988) compared $NaHCO_3$ (ALK) and NaCl (PLA) against a control trial (CON) on 5 x 100 yard intervals swims (2 mins rest between each swim) in well-trained swimmers. Performance times for swims 4 and 5 were quicker for ALK compared to PLA and CON. Likewise, Lindh et al. (2008) reported that 200 m swimming performance was faster in elite swimmers after $NaHCO_3$ compared to CON and PLA. Similarly, during 4 x 50 m front crawl sprints interspersed by 1 min passive recovery, total swim time and swimming speed during the first 50 m sprint, respectively, were faster for $NaHCO_3$ compared to PLA (Zajac et al. 2009).

However, not all research examining $NaHCO_3$ on swimming performance has demonstrated ergogenic benefit. Pierce et al. (1992) reported that $NaHCO_3$ had no effects on 100 yard (as part of relay) or 200 yard (solo) swimming performance. In the research conducted by Pruscino et al. (2008), six elite male swimmers completed 2 maximal 200 m freestyle swims with 30 mins passive rest after consuming either 0.3 $g.kg^{-1}$ $NaHCO_3$, 6.2 $mg.kg^{-1}$ caffeine, a combination of $NaHCO_3$ and caffeine or PLA (glucose). The authors reported no significant difference in mean performance time between all four treatments for both maximal swims. However, closer analysis revealed moderate effect sizes of 0.6 and 0.8 when comparing the $NaHCO_3$-caffeine combination to PLA. Moreover, effect sizes of 0.2 and 0.4 were evident when comparing $NaHCO_3$ to PLA suggesting that ergogenic benefit for the $NaHCO_3$-caffeine combination and $NaHCO_3$ alone were demonstrated, at least in some individuals, compared to PLA (Pruscino et al. 2008).

2.3.7 Other physiological responses

A number of studies have evaluated the potential beneficial physiological effects of $NaHCO_3$ on cycling (Table 2.2). More simply, rather than focussing purely on exercise performance / capacity researchers have looked at the effects of $NaHCO_3$ on other physiological parameters such as $\dot{V}O_2$ kinetics (Kolkhorst et al. 2004, Berger et al. 2006), EMG activity (Kostra and Cafarelli 1982, Yamanaka et al. 2011), muscle fibre conduction (Hunter et al. 2009), vascular endothelial growth factor (VEGF) production (cytokines involved in angiogensis; Wahl et al. 2011), metabolic distribution (Galloway and Maughan 1996) and serum prolactin production (Vega et al. 2006). Due to the limited research on $NaHCO_3$ and its effects on serum prolactin production (Vega et al. 2006), VEGF production (Wahl et al. 2011) and metabolic distribution (Galloway and Maughan 1996) it is difficult to compare and contrast results and thus properly evaluate the efficacy of $NaHCO_3$ on these parameters. Therefore, the remainder of this section will examine research into possible beneficial physiological effects on $\dot{V}O_2$ kinetics (Kolkhorst et al. 2004, Berger et al. 2006) and EMG activity (Kostra and Cafarelli 1982, Yamanaka et al. 2011).

$\dot{V}O_2$ kinetics can be divided into cardio-dynamic, rapid and slow components (Kolkhorst et al. 2004; Figure 2.6) although there can be slight variations of these definitions within the literature. Kolkhorst et al. (2004) reported that $NaHCO_3$ ingestion slowed the rapid component of $\dot{V}O_2$ kinetics by 25% and decreased the amplitude of the slow component (A_3) by 29%. The authors suggested that the reduction in amplitude of A_3 was due to diminished fatigue which could help to explain, at least in part, why ergogenic benefits have been observed in high-intensity exercise. Based on observations from previous research that acidemia might increase muscle perfusion during heavy exercise, Kolkhorst et al. (2004) speculated that the slowing of the rapid component was due to reduced perfusion in working muscle. It was subsequently speculated that O_2 delivery might limit mitochondrial respiration at the onset of high-intensity exercise. Berger et al. (2006) reported that $NaHCO_3$ ingestion significantly reduced pulmonary $\dot{V}O_2$ ($p\dot{V}O_2$) during the slow component after 6 mins of exercise but also that the $p\dot{V}O_2$ slow component occurred ~ 23% later (147 vs. 120 sec).

Although the amplitude of the slow component (A_3) was reported as not being significantly different (P = 0.08), A_3 for $NaHCO_3$ was 19% lower than CON (ES = 0.8). Therefore, in contrast to the reported results this suggests $NaHCO_3$ did decrease the amplitude of the slow component in this study to a similar level observed by Kolkhorst et al. (2004).

Figure 2.6 A three-component exponential model demonstrating cardio-dynamic (1), rapid (2), and slow (3) components of $\dot{V}O_2$ kinetics; Where $\dot{V}O_{2base}$ is the steady-state $\dot{V}O_2$ at the onset of exercise and A_1, A_2, and A_3 are amplitudes of each component (Kolkhorst et al. 2004).

Interestingly, Zoladz et al. (2005) reported that $NaHCO_3$ ingestion significantly reduced time spent during the fast-component by ~ 25% compared to PLA at 87% $\dot{V}O_{2MAX}$ although no difference was observed at 40% $\dot{V}O_{2MAX.}$ Therefore, it's plausible that $\dot{V}O_2$ kinetics are only modulated by $NaHCO_3$ ingestion above a certain threshold of exercise intensity. According to Zoladz et al. (2005) this might be due to changes in the rate of oxidative phosphorylation in the working muscles, presumably a rate increase which would theoretically lessen the demand on anaerobic glycolysis from type II muscles which are

utilised extensively during high-intensity exercise. However, muscle metabolites were not measured by Zoladz et al. (2005) and therefore more research is required to elucidate changes in $\dot{V}O_2$ kinetics with $NaHCO_3$ ingestion at different exercise intensities. In contrast, not all studies demonstrate an improvement in $\dot{V}O_2$ kinetics with $NaHCO_3$ ingestion. Santalla et al. (2003) reported no difference in the amplitude of the $\dot{V}O_2$ slow component in 2 x 6 mins cycling at 90% $\dot{V}O_{2MAX}$. However, as described in section 2.3.5 it is plausible that the elite status of the participants in the Santalla et al. (2003) study might be one reason why their results differ to Kolkhorst et al. (2004) and Berger et al. (2006).

According to Yamanaka et al. (2011) the use of electromyography (EMG) and perceived sense of effort can provide an estimate of central motor command during exercise. Yamanaka et al. (2011) reported that $NaHCO_3$ ingestion had no effects on integrated EMG (iEMG), effort sense in the legs or ventilatory response during or after 2 mins intense cycling exercise. Similarly, Kostra and Cafarelli (1982) found that there was no difference in iEMG or sensory response between $NaHCO_3$ and PLA trials. However, sensory activity was greater for the acidotic trial (NH_4Cl) compared to $NaHCO_3$ and PLA suggesting that sensory activity might be influenced by changes in acid-base balance. Finally, despite no differences in EMG activity Hunter et al. (2009) reported significantly faster muscle fibre conduction velocity after $NaHCO_3$ compared to PLA ($CaCO_3$) concomitant with a reduced rate of force decline. It was suggested that an increase in extracellular pH alters the sarcolemma to increase muscle fibre conduction velocity. Therefore, it is possible that $NaHCO_3$ increases muscle fibre conduction velocity which attenuates force decline through attenuation of neuromuscular fatigue (Hunter et al. 2009). In turn this might subsequently attenuate self-reported sensory responses and potentially $\dot{V}O_2$ kinetics.

2.3.8 Perceptual responses

A number of perceptual responses such as subjective ratings of fatigue or effort (Poulus et al. 1974, Swank and Robertson 1989, 2002, Galloway and Maughan 1996, Yamanaka et al. 2011) and perceived readiness to exercise (Zabala et al. 2008, 2011) have also been evaluated in relation to potential beneficial psychological effects of $NaHCO_3$ on cycling (Table 2.2). For example, Poulus et al. (1974) demonstrated that ~ 0.3 $g.kg^{-1}$ $NaHCO_3$ had no effect on the subjective feeling of fatigue of trained male subjects undertaking an incremental cycling to exhaustion. However, as the $NaHCO_3$ was administered intravenously, this may have contributed to the results. In contrast, Swank and Robertson (1989) highlighted that differentiated RPE for legs (RPE_L), chest (RPE_C) and whole body (RPE_{WB}) were significantly lower during 3 x 5 mins cycling (10 mins rest between bouts) at 90% $\dot{V}O_{2\ MAX}$ after a 0.3 $g.kg^{-1}$ $NaHCO_3$ bolus (ALK-B) and staggered administration (ALK-S; 0.12 $g.kg^{-1}$) compared to PLA. Whole body RPE was also lower after the ALK-B compared to ALK-S administration although there were no differences in RPE_C and RPE_L. In the same participant cohort, although published 13 years later, Swank and Robertson (2002) reported that recovery of RPE_L was 8% greater for ALK-B compared to ALK-S and PLA at 1 and 2 mins during post-exercise recovery. Additionally, after 2 mins recovery of RPE_{TB} was 9% greater compared to ALK-S and PLA. However, some caution should be applied when interpreting these results. Swank and Robertson (1989, 2002) state that the participants were endurance trained females. However, the mean $\dot{V}O_{2MAX}$ of 43.5 ± 5.0 $ml.kg^{-1}.min^{-1}$ suggests a low trained cohort. As such the results highlight a benefit of using $NaHCO_3$ in similar populations, rather than endurance trained female cyclists. Furthermore the staggered $NaHCO_3$ dosage used in ALK-S did not augment acid-base balance as much as the ALK-B trial which is likely to have impacted results (Swank and Robertson 1989, 2002). Price, Moss and Rance (2003) highlighted no difference in RPE between trials of 30 mins of intermittent cycling exercise and Yamanaka et al. (2011) reported no differences during 2 mins intense cycling. In contrast, Galloway and Maughan (1996) found that RPE was greater after 40 mins cycling at ~ 70% $\dot{V}O_{2\ MAX}$ with $NaHCO_3$ compared to PLA. In order to help clarify the inconsistent results in this area we examined the effects of $NaHCO_3$ on

differentiated RPE during high-intensity cycling at different exercise intensities in the same population (chapter 5) and before and after 6 weeks high intensity training (chapter 7).

Overall, 56% (9 out of 16) of studies examining cycling performance showed a physiological or perceptual benefit with exogenous $NaHCO_3$ supplementation during cycling (Table 2.2). However, when 'optimal' research characteristics, as outlined previously are met this increases to 75% (3 out of 4 studies). In contrast physiological or perceptual benefit dropped to 50% when only 2 (4 out of 8 studies) or 1 (2 out of 4 studies) of the outlined optimal characteristics outlined were employed. In summary, there appears to be a relationship between the chances of observing a physiological or perceptual benefit in a cycling protocol and the number of 'optimal' research characteristics already outlined having been met. However, this relationship appears less clear than for performance / capacity based research which might be expected due to the variety of physiological or perceptual mechanisms being examined whereas exercise performance / capacity research is arguably more homogenous to interpret.

Table 2.2 Summary of research that has evaluated the potential mechanistic effects of $NaHCO_3$ on cycling

Author(s)	$NaHCO_3$ Dosage ($g.kg^{-1}$ body mass)	Protocol	n	Results	Benefit
Poulus et al. (1974)	~ 0.3 $g.kg^{-1}$	4 x incremental exercise tests (10 W.min-1) under 3 conditions: (1) ALK ($NaHCO_3$), (2) PLA (NaCl) and CON (no infusion)	6 trained males	ALK had no effect on subjective ratings of fatigue during exercise	No
Kostra and Cafarelli (1982)	0.3 $g.kg^{-1}$	15 mins at 50% $\dot{V}O_{2\,MAX}$, followed by 15 mins at 80% $\dot{V}O_{2\,MAX}$ after: (1) $NaHCO_3$ (ALK), (2) $CaCO_3$ (PLA) and (3) NH_4Cl (ACD)	6 males	There was no difference in integrated EMG (iEMG) or sensory response between ALK and PLA. However, sensory activity was greater for ACD compared to PLA and ALK	No
Swank and Robertson (1989)	0.3 $g.kg^{-1}$ or 0.12 $g.kg^{-1}$ and 0.18 $g.kg^{-1}$ prior to exercise	3 * 90% $\dot{V}O_{2\,MAX}$ for 5 mins (10 mins rest between) after: (1) $CaCO_3$ (PLA), (2) $NaHCO_3$-Bolus (ALK-B) or (3) $NaHCO_3$-Staggered (ALK-S). 0.06 $g.kg^{-1}$ $CaCO_3$ also administered prior to exercise in PLA and ALK-B to blind against ALK-S	6 trained females	RPE for Legs (L), Chest (C) and Total Body (TB) were significantly lower for ALK-B and ALK-S compared to PLA. RPE-TB was also lower for ALK-B compared to ALK-S although there were no differences in C and L	Yes
Galloway and Maughan (1996)	0.3 $g.kg^{-1}$	1-h cycling at ~ 70% $\dot{V}O_{2\,MAX}$ after: (1) $NaHCO_3$ (ALK) and (2) $CaCO_3$ (PLA)	7 healthy males	BLa significantly greater during ALK at all time points. No differences in any other metabolites were observed (glucose, glycerol, FFA). RPE and $\dot{V}O_2$ were also greater for ALK than PLA	No
Neilsen et al. (2002)	Unclear	5 mins handgrip exercise, 2 s at 40% MVC, followed by 1 s after: (1) $NaHCO_3$ (ALK) and (2) Saline (PLA) via IV administration	9 healthy males	IV infusion of $NaHCO_3$ attenuated the reduction in pHi at the end of exercise where pHi was at its nadir	Yes
Swank and Robertson (2002)	0.3 $g.kg^{-1}$ or 0.12 $g.kg^{-1}$ and 0.18 $g.kg^{-1}$ prior to exercise	3 * 90% $\dot{V}O_{2\,MAX}$ for 5 mins (10 mins rest between) after: (1) $CaCO_3$ (PLA), (2) $NaHCO_3$-Bolus (ALK-B) or (3) $NaHCO_3$-Staggered (ALK-S). 0.06 $g.kg^{-1}$ $CaCO_3$ also administered prior to exercise in PLA and ALK-B to blind against ALK-S	6 females	Average recovery of RPE_L and RPE_C was 8% greater for ALK-B compared to ALK-S and PLA at 1 and 2 mins during recovery. Additionally, RPE_O was 10% greater after 1 min recovery compared to PLA and RPE_O was 9% greater after 2 mins recovery compared to ALK-S and PLA	Yes
Santalla et al. (2003)	0.3 $g.kg^{-1}$	2 * 90% $\dot{V}O_{2\,MAX}$ for 6 mins (8 mins active recovery) after: (1) $NaHCO_3$ (ALK) or (2) PLA	7 elite cyclists	ALK did not attenuate the $\dot{V}O_2$ slow component	No
Kolkhorst et al. (2004)	0.3 $g.kg^{-1}$	6 mins bout 25 W above ventilatory threshold after: (1) $NaHCO_3$ (ALK), (2)H_2O only (CON)	9 males, 1 female	ALK slowed the rapid component of $\dot{V}O_2$ kinetics and also decreased the amplitude of the slow component	Yes

Author(s)	$NaHCO_3$ Dosage ($g.kg^{-1}$ body mass)	Protocol	n	Results	Benefit
Zoladz et al. (2005)	0.25 $g.kg^{-1}$	2 x 6 mins (40% $\dot{V}O_{2MAX}$ and 87% $\dot{V}O_{2MAX}$ interspersed by 20 mins recovery) after (1) $NaHCO_3$ (ALK) or placebo (PLA)	7 males	ALK significantly reduced time spent during fast-component compared to PLA at87% $\dot{V}O_{2MAX.}$ No difference was observed at 40% $\dot{V}O_{2MAX}$	Yes
Berger et al. (2006)	0.3 $g.kg^{-1}$	3 mins at 20 W, then 6 mins step test at ~ 80% $p\dot{V}O_{2MAX}$ (283 +/- 31 W) after: (1) $NaHCO_3$ (ALK), (2) NaCl (PLA) on 3 occasions (i.e. 6 tests)	7 males	ALK significantly reduced $p\dot{V}O_2$ during the $p\dot{V}O_2$ slow component after 6 mins of exercise. The $p\dot{V}O_2$ slow component was delayed longer with ALK (147 vs. 120 s; ~ 23% longer)	Yes
Vega et al. (2006)	Unclear	Incremental exercise after: (1) $NaHCO_3$ (ALK) and (2) Saline (CON)	7 males (active)	No difference in T_{LIM} between ALK and CON. However, post-exercise ALK attenuated the increase of serum prolactin production	Yes
Siegler et al. (2008)	0.3 $g.kg.^{-1}$	T_{LIM} at 120% $\dot{V}O_{2\,MAX}$ after: (1) $NaHCO_3$ (ALK) and (2) $CaCO_3$ (PLA). Recovery was monitored after each trial in passive/active forms (total of 4 trials)	9 males	ALK attenuated post-exercise acid-base recovery more than PLA regardless of whether active or passive recovery was undertaken (although ALK-Active is probably most efficient)	Yes
Zabala et al. (2008)	0.3 $g.kg.^{-1}$	3 x 30 s WAnT (30 mins recovery after: (1) $NaHCO_3$ (ALK) or (2) NaCl (PLA)	9 elite BMX riders	Perceived readiness to exercise improved in ALK prior to WAnT 2 and 3 (although no ergogenic benefit was subsequently observed)	Yes
Hunter et al. (2009)	0.3 $g.kg^{-1}$	MVC was measured pre and post 1 hour of submaximal cycling at 105% of LT after: (1) $NaHCO_3$ (ALK) and (2) $CaCO_3$ (PLA). MVC pre-exercise with electrical stimulation whereas MVC post-exercise undertaken with and without (sustained) stimulation	8 trained cyclists	Muscle fibre conduction measured during sustained (50 s) MVC was greater for ALK compared to PLA. Additionally muscle fibre conduction was greater post-ingestion of ALK than pre-ingestion which might help to prevent force decline indicating better fatigue resistance (i.e. attenuation of neuromuscular fatigue)	Yes
Zabala et al. (2011)	0.3 $g.kg.^{-1}$	3 x 30 s WAnT (15 mins recovery) after: (1) $NaHCO_3$ (ALK) or (2) NaCl (PLA)	10 elite BMX riders	There were no differences in perceived readiness to exercise between WAnT trials	No
Yamanaka et al. (2011)	0.3 $g.kg.^{-1}$	2 mins at 105-110% of W_{MAX} after: (1) $NaHCO_3$ (ALK) or (2) $CaCO_3$ (PLA). Warm up consisted of 6 mins at 20 W and recovery was 30 mins at 20 W	6 males	ALK did not affect integrated EMG (iEMG), effort sense in the legs or ventilatory response during or after 2 mins intense cycling exercise	No
Wahl et al. (2011)	0.3 $g.kg.^{-1}$	4 x 30 s all-out efforts separated by 5 mins passive recovery and 60 mins at 50% PPO after: (1) $NaHCO_3$ (ALK) or (2) $CaCO_3$ (PLA)	11 males	ALK had no effect on VEGF compared to PLA	No

2.3.9 Gastrointestinal discomfort

One of the biggest disadvantages of $NaHCO_3$ supplementation is that of GI discomfort (Burke and Pyne 2007). Symptoms can include nausea, stomach pain, diarrhoea and occasionally vomiting (Kolkhorst et al. 2004, Carr et al. 2011). Such effects are a serious practical consideration for individuals, especially for athletes in a competitive setting (Burke and Pyne 2007). A number of studies have reported GI distress after consumption of $NaHCO_3$ (McNaughton, Ford, and Newbold 1997, Kolkhorst et al. 2004, Cameron et al. 2010, Saunders et al. 2011, Driller et al. 2012b). McNaughton, Ford, and Newbold (1997) observed GI distress in 30% of their participants (3/10) when consuming the 'optimal' dose of $0.3g.kg^{-1}$, although overall group performance was still improved after $NaHCO_3$. Similarly, Price, Moss, and Rance (2003) reported an ergogenic benefit even though gut fullness (GF) and abdominal discomfort (AD) were significantly higher for $NaHCO_3$ compared to PLA. In contrast, Cameron et al. (2010) highlighted that the severity and incidence of GI symptoms in elite rugby players might have been a major contributor to the lack of any performance improvement during a rugby specific protocol. Similarly, Saunders et al. (2011) found no difference in total work done (TWD) T_{LIM} at 110% W_{PEAK} for group data. However, when participants with GI distress were removed from the analysis (n=4), TWD was ~ 5% greater for $NaHCO_3$ compared to PLA. This led the authors to classify participants as either responders or non-responders to $NaHCO_3$ (Price and Simons 2010, Saunders et al. 2011). This aspect is addressed in study 2 of this thesis (chapter 5) from both capacity (i.e. T_{LIM}), perceptual (AD, GF, RPE etc.) and physiological (BLa, pH, HR etc.) responses.

Carr et al. (2011) analysed different ingestion protocols by varying fluid intake, ingestion timing, co-ingesting a small meal and using either capsules or a solution for $NaHCO_3$ ingestion. The authors suggest substantial blood alkalosis is achieved 120-150 mins before exercise (presumably at the end of the staggered ingestion) and GI symptoms reduced when $NaHCO_3$ was co-ingested with a high carbohydrate meal. However, as

performance was not measured, and presumably some enhanced tolerance of $NaHCO_3$ occurred within the crossover design, more research is required to substantiate these results. The reduction of GI discomfort after combined $NaHCO_3$-carbohydrate intake is supported by Price and Cripps (2012) who reported that ingesting a combined $NaHCO_3$-carbohydrate beverage resulted in lower AD and GF after 15 mins absorption compared to the $NaHCO_3$-only beverage, although no difference in performance was observed. Interestingly, the absorption period used by Price and Cripps (2012) was 60 mins and carbohydrate was ingested as a solution rather than a meal (Carr et al. 2011) and thus further research is required to fully elucidate the effects of combined $NaHCO_3$-carbohydrate ingestion on exercise performance.

In order to prevent GI distress affecting competitive performance individuals should attempt a trial period, prior to competitive performance, to determine if habituation to $NaHCO_3$ lessens these issues and facilitates ergogenic benefit (McNaughton, Ford, and Newbold 1997, Cameron et al. 2010). This would appear especially important for mesomorphs with higher than typical athletic body mass (i.e. > 85kg; Cameron et al. 2010). One method that might ameliorate potential GI discomfort is that of $NaHCO_3$ 'loading' where individuals consume $NaHCO_3$ over several days or consume multiple acute doses prior to exercise, as opposed to a one-off acute dose (Burke and Pyne 2007). Such approaches are reviewed in section 2.3.10.

2.3.10 Loading regime

Most of the research evaluating $NaHCO_3$ as an ergogenic aid has used acute supplemental boluses. However, McNaughton et al. (1999) demonstrated that after 5-days $NaHCO_3$ supplementation (0.5 $g.kg^{-1}$ per day consumed in 4 equal amounts) work done (TWD) and peak power (PPO) increased during 60 s maximal work by 10% and 14% and 10% and 14%, respectively, when compared to pre-supplementation and CON (i.e. after

$NaHCO_3$ washout) trials. The authors suggested that the body stored the extra bicarbonate and utilised this to enhance performance during the 60 s maximal cycling exercise. However, [HCO_3^-], pH, and base excess did not change after their initial significant increases (24h) which led the authors to suggest that similar ergogenic benefits might be observed with an ingestion period of less than 5-days. Moreover, the values achieved of ~ 28 mM, 7.45 and 10.42 for [HCO_3^-], pH, and base excess, respectively, are similar to those usually seen with acute administration of 0.3 $g.kg^{-1}$ $NaHCO_3$.

Similarly, McNaughton and Thompson (2001) examined 90 s cycling performance after either an acute dose (0.5 $g.kg^{-1}$) or chronic ingestion (0.5 $g.kg^{-1}$ per day for 6 days) of $NaHCO_3$. Cycling performance was measured on day 1 (CON), day 7 (last day of supplementation for chronic ingestion group and ingestion for acute group) and days 8 and 9 (no ingestion). Here, $NaHCO_3$ ingestion facilitated greater work done compared to PLA in both $NaHCO_3$ conditions although there was more work done (compared to CON) on the final 2 days (i.e. days 8 and 9) in the chronic condition. Therefore, chronic $NaHCO_3$ ingestion can improve work done two days after ingestion has ceased (McNaughton and Thompson 2001). In a similar experiment to McNaughton et al. (1999), Douroudos et al. (2006) demonstrated that MPO during 30 s WAnT was significantly greater after 5-days of 0.5 $g.kg^{-1}$ per day of $NaHCO_3$ compared to both 0.3 $g.kg^{-1}$ per day $NaHCO_3$ and CON groups. Therefore although $NaHCO_3$ loading might improve subsequent short duration high-intensity exercise performance a threshold of 0.5 $g.kg^{-1}$ $NaHCO_3$ per day seems to exist when ingestion over a period of 1-day or longer is considered.

In a study by Bishop and Claudius (2005) participants ingested two x 0.2 $g.kg^{-1}$ $NaHCO_3$ or 0.138 $g.kg^{-1}$ NaCl (PLA), 90 and 20 minutes before prolonged intermittent cycling. Cycling consisted of two ~ 36 minute periods of 18 x ~ 2 minute blocks which included a 4 s all out sprint, 100 seconds active recovery at 35% $\dot{V}O_{2PEAK}$ and 20 s rest. This loading regime increased plasma [HCO_3^-] by 5.5 $mmol.l^{-1}$ which is similar to that reported

for a single 0.3 $g.kg^{-1}$ dose of $NaHCO_3$ (5.3 $mmol.l^{-1}$; Matson and Tran 1993). Moreover, more work was completed in 7 out of 18 sprints after staggered $NaHCO_3$ ingestion during the second period of the exercise protocol designed to mimic the intermittent nature of field hockey (Bishop and Claudius 2005). These results suggest that 'stacking' $NaHCO_3$ loads could be utilised by those who suffer GI disturbances with a single $NaHCO_3$ bolus. However, it should be noted that higher increases of [HCO_3^-] (6.9 $mmol.l^{-1}$) have also been observed with single 0.3 $g.kg^{-1}$ doses of $NaHCO_3$ (Siegler et al. 2008), although variation in administration timing will account, at least in part, for difference observed between studies. Recent research by Siegler et al. (2010) evaluated the time course of acid-base balance over 2 hours after 0.1 $g.kg^{-1}$, 0.2 $g.kg^{-1}$ and 0.3 $g.kg^{-1}$ $NaHCO_3$. Base excess, pH and [HCO_3^-] peaked after ~ 50 and 65 mins for 0.2 $g.kg^{-1}$ and 0.3 $g.kg^{-1}$ $NaHCO_3$, respectively. The results presented by Siegler et al. (2010), which are supported by Renfree (2007) and Price and Singh (2008), further ratify the choice of a 60 mins ingestion period for the human studies in this thesis.

2.3.11 Placebo effect

McClung and Collins (2007) evaluated the effects of $NaHCO_3$ on 1000 m running performance under 4 experimental conditions. Participants ingested either 0.3 $g.kg^{-1}$ $NaHCO_3$ or a PLA (NaCl) and were told that they were receiving $NaHCO_3$ or they were not receiving $NaHCO_3$. Interestingly, although the 'Told/Given' condition resulted in the fastest 1000 m running performance, the 'Told/Not Given' condition performed better than the 'Not Told/Given' and 'Not Given/Not Told' conditions by ~ 1.8 % (3.4 s) and 1.5 % (2.8 s). Based on the average running speed for all conditions of 5.4 $m.s^{-1}$ this equates to ~ 18 m and ~ 15 m respectively, very possibly the difference between first and last place in a competitive race. Therefore, in isolating the potential psychological effects (i.e. 'Told/Not Given') the authors demonstrate that factors such as ergogenic expectancy might help athletes out-perform those who take ergogenic aids but don't have such expectancy (i.e. Not Told/Given).

More simply, McClung and Collins (2007) have demonstrated that psychological factors (i.e. the placebo effect) might play a role in whether an ergogenic benefit is observed with $NaHCO_3$. However, it's important to point out that such expectancy has been eliminated from this thesis as much as possible by blinding participants to experimental solutions and by taste matching them as near as possible. Moreover, recent research using a similar endurance trained cohort to that of McClung and Collins (2007), albeit in cycling, demonstrated that during 2 min maximal cycling MPO and PPO were significantly greater for $NaHCO_3$ compared to PLA (NaCl) and CON (Driller et al. 2012a). Additionally, McClung and Collins (2007) only used a 'pinch' of NaCl (exact amount unclear) as the PLA which might have caused taste matching issues which theoretically might have impacted on results. More research evaluating the possible psychological effects on performance, such as placebo, is warranted (Beedie 2007).

2.4 The effects of $NaHCO_3$ on isolated muscle performance

In an attempt to clarify the effects of perturbations in acid-base balance at a tissue level several studies have examined the effects of metabolic alkalosis and acidosis on isolated muscle performance. Such an approach is useful in assessing mammalian muscle function as this eliminates the role of central fatigue (Allen, Lamb, and Westerblad 2008) which can be affected by numerous factors such as mood or emotional state, intrinsic/extrinsic motivation and/or pre-experimental nutritional status. By isolating the muscle, scientists can examine direct muscular responses which might provide valuable mechanistic evidence as to how the imposed intervention affects muscle function during exercise performance. The literature evaluating the effects of $NaHCO_3$ on isolated muscle performance will be split into amphibian and mammalian muscle categories due to the biological differences between species which could impact on whether $NaHCO_3$ affects muscle performance.

2.4.1 Amphibian muscle

Mainwood and Lucier (1972) examined the time course of recovery in isolated frog sartorius muscles superfused in Ringer's solution. After 200 s electrical stimulation (400 impulses at 2.s^{-1}) isometric (muscle held at constant length) twitch tension fell to 10–30% of its resting value. When the muscle was superfused in Ringer's solution of 25 mmol.l^{-1} [HCO_3^-] tension measured 80–90% of resting values at the end of 60 mins recovery. In contrast, when the solution was reduced to 1 mmol.l^{-1} [HCO_3^-] isometric twitch tension recovered to only 30–40% of resting values (Mainwood and Lucier 1972). Using the same protocol Mainwood, Worsley-Brown and Paterson (1972) examined the metabolic changes in frog sartorius muscle. The authors reported that muscle lactate remained elevated after 75 mins recovery in the 1 mmol.l^{-1} [HCO_3^-] solution but that muscle lactate levels had returned to almost resting levels in the 25 mmol.l^{-1} [HCO_3^-] solution. This was suggested to be largely due to greater efflux of lactate with the peak rate of lactate efflux being 133% greater in the 25 mmol.l^{-1} [HCO_3^-] solution. Interestingly, Mainwood and Worsley-Brown (1975) found that the efflux of lactate was reduced further for muscles superfused in 1 mmol.l^{-1} buffer (imidazole) solution with potassium sulphate induced depolarisation. The rate of lactate efflux did drop slightly (5-10%) in muscles superfused in the 25 mmol.l^{-1} buffer solution but this was still 100% greater than the rate of lactate efflux in muscles superfused in 1 mmol.l^{-1} buffer (imidazole) solution. Therefore, efflux of lactate might also be dependent on membrane potential in addition to buffer perfusate concentration (Mainwood and Worsley-Brown 1975).

The effect of low (6.6) and high (7.9) extracellular pH (pH_e) on power output in frog sartorius muscle has also been examined by adjusting pCO_2 as opposed to muscle perfusate (Stevens 1988). This study, which used the work-loop technique (as described in chapter 6), reported that mass specific maximum power (i.e. W.kg^{-1}) was ~ 25% lower at low pH_e. However, some caution should be applied when interpreting these results. For

example, it is unlikely that the reported levels of pH_e would be achieved *in vivo* (certainly in humans) and the physiological perfusate used was 5 mM lower in $NaHCO_3$ (i.e. 20 mM) than reported previously. Nevertheless, these results support the result previously presented suggesting that the physiological concentration of the extracellular buffer fluid and/or associated pH is likely to have substantial effects on amphibian skeletal muscle (Mainwood and Lucier 1972, Mainwood, Worsley-Brown and Paterson 1972, Mainwood and Worsley-Brown 1975).

2.4.2 Mammalian muscle

Hirche et al. (1974) examined the effects of changes in acid-base homeostasis on the rate of lactic acid permeation from isolated dog gastrocenemii. Metabolic acidosis (ACD) and metabolic alkalosis were induced by infusions of hydrochloric acid (HCl; ACD) and $NaHCO_3$ (ALK-S) or trishydroxymethylamino-methane (ALK-T), respectively. The authors reported that lactate efflux was ~ 150% greater for ALK-T and ACT-S compared to ACD supporting the work by Mainwood, Worsley-Brown and Paterson (1972) who found similar results in frog muscle.

Recognising that experiments on amphibian muscle can not necessarily be translated to mammalian muscle, Mainwood and Cechetto (1980) evaluated the effects of bicarbonate concentration on fatigue and recovery in isolated rat diaphragm muscle. Muscles were incubated for 30 mins in solutions of 2, 10 or 25 mM [HCO_3^-] and were subjected to 24 supramaximal pulses (i.e. 0.2 s at 120 Hz) once a minute for 30 mins. Fatigue was then induced by increasing the train frequency to 2 Hz. Once fatigue had developed (~ 3 mins) the train frequency returned to pre-fatigue levels for 30 mins recovery. Experiments were completed at both 30°C and 37°C. The authors reported that isometric tension reduced to ~ 25% of pre-fatigue control values regardless of experimental solution or temperature. However, after ~ 6 mins recovery isometric tension recovered ~ 100% with the

25 mM [HCO_3^-] solution whereas recovery was ~ 50% for the 2 mM [HCO_3^-] solution. At the same time point isometric tension recovered ~ 75-90% for the 10 mM [HCO_3^-] solution. Interestingly, patterns of recovery were largely similar regardless of temperature although generally higher at 37°C (Mainwood and Cechetto 1980).

Spriet et al. (1985) induced metabolic acidosis by lowering the [HCO_3^-] in the isolated muscle perfusate from ~ 24 mM to ~ 13 mM. Metabolic acidosis significantly increased the rate of muscle tension decay and reduced absolute muscle tension in the gastrocnemius-plantaris-soleus muscle group of rats, during fatiguing isometric stimulation, compared to the ~ 24 mM solution. Conversely, Spriet et al. (1986) found that inducing metabolic alkalosis by increasing the [HCO_3^-] to ~ 27 mM had no effect on peak isometric tension or tension decay compared to CON (~ 21 mM) in the gastrocnemius-plantaris-soleus muscle group of rats. Finally, Broch-Lips et al. (2007) examined the effect of 40mM and 25mM [HCO_3^-] on isometric force production in isolated rat skeletal muscle. The elevated HCO_3^- had no significant effect on force maintenance during continuous stimulation or recovery of force during brief tetanic stimulation in soleus or on tetanic force development in extensor digitorum longus muscles at 30°C. Similarly, 40 mM of HCO_3^- had no significant effect on isometric force maintenance during either continuous stimulation or intermittent stimulation protocols (1 s on, 3 s off) at 37°C (Broch-Lips et al. 2007).

It is important to acknowledge that metabolic acidosis or alkalosis induced in the extracellular space, through modulation of [HCO_3^-], is not the sole component of acid-base balance that might impact on isolated muscle performance. Indeed, Wetzel et al. (2001) report that extracellular carbonic anhydrase plays a critical role in both H^+ and lactate transport in rat skeletal muscle. In contrast, intracellular carbonic anhydrase did not contribute to H^+ and lactate kinetics, which was posited to be because of the high concentration of non-HCO_3^- buffers in the intracellular space. Similar to the work by Stevens (1988), Lannergren and Westerblad (1991) examined the effects of modulating acid-base

balance in flexor brevis feet muscle in mice by increasing CO_2. The authors reported that tetanic tension reduced to ~ 80% of maximum further demonstrating the complex nature of acid-base balance and muscle performance.

Overall the data examining the effects of acid-base changes on both amphibian and mammalian muscle suggest an important role for [HCO_3^-] in terms of muscle performance, possibly through facilitating efflux of lactate/H^+ and/or maintenance of an optimal physiological milieu for cellular functioning (i.e. pH). However, it's important to acknowledge that not all studies report that lactic acid/lactate have a detrimental impact in isolated muscle performance. Indeed, Nielsen, de Paoli, and Overgaard (2001) report that in rat soleus muscles lactic acid had protective effects on muscle excitability and force against increases in extracellular potassium (K^+_e) which has been suggested to contribute to muscle fatigue (Bangsbo et al. 1996, Bangsbo and Juel 2006). This was demonstrated by adding lactic acid to the perfusate which also had levels of K^+_e (11 mM) usually seen in skeletal muscle during intense exercise. By adding lactic acid recovery of force increased from ~ 30 % to ~ 100% of control values (4 mM K^+_e). However, as Nielsen, de Paoli, and Overgaard (2001) did not adjust extracellular [HCO_3^-] it is unclear how this might further affect isolated muscle performance.

Although the aforementioned *in vitro* studies have examined the effects of high and low [HCO_3^-] on muscle performance the current body of isolated muscle research has a number of methodological concerns. For example, during mammalian locomotion muscles that are attached to moving skeletal structures, either directly or indirectly, undergo repetitive length changes (Josephson 1993). Approximation of such length changes *in vitro* facilitates the evaluation of important components of exercise performance such as recovery from fatigue (James, Wilson, and Askew 2004) as well the possible direct effects of ergogenic aids (Tallis et al. 2012) in mammalian muscle. As such, research using isometric muscle protocols has limited application to muscle performance during dynamic exercise which is

exhibited in many muscles during mammalian locomotion. Moreover, to the best of our knowledge only one study has examined the effects of changes in acid-base balance at an isolated muscle level using a protocol that mimics cyclical length changes that occur in locomotion (Stevens 1988). However, acid-base balance was modulated by adjusting pCO_2 as opposed to extracellular perfusate. Moreover, the physiological perfusate used was lower than observed in typical resting levels (i.e. 20 vs. 25mM) and the muscle used was amphibian rather than mammalian. Furthermore, no research reported to date examining acid-base balance at a tissue level has used levels of [HCO_3^-] that are typically achieved in the blood of human participants (~32mM; Kolkhorst et al. 2004, Price and Singh 2008, Lindh et al. 2008, Siegler et al. 2010) following the recommended supplementation dosage (0.3 $g.kg^{-1}$; McNaughton 1992a). Therefore, *in vitro* research that addresses these key gaps would provide useful data as to how augmented [HCO_3^-] might affect human exercise performance / capacity (study 3, chapter 6).

Chapter 3 – General Methods

This chapter outlines the common experimental methods used within this thesis. A more detailed description of specific methods completed for each individual study is described in the appropriate chapter.

3.1 Ethics

University ethics approval was applied for and obtained for each individual study. Before taking part in each study, human participants were supplied with a document that outlined the purpose of the proposed research, how the study would be carried out and what would be expected of them, should they agree to participate. If participants were agreeable to the suggested research, they were then provided with an informed consent form which they completed and signed. Each participant was free to withdraw from the study at any stage.

The study examining the effects of $NaHCO_3$ on isolated muscle performance (chapter 6) was carried out in accordance with the British Home Office Animals (Scientific Procedures) Act 1986, Schedule 1.

3.2 Participation screening

Participants were requested to avoid caffeine for at least 12 hours prior to exercise, alcohol and strenuous exercise for at least 24 hours prior to exercise and to adopt the same mixed balanced diet on each testing day. For studies involving oral ingestion of $NaHCO_3$ (chapters 5 and 7) participants were specifically requested to avoid low carbohydrate intake which may induce mild metabolic acidosis which can negatively affect high-intensity exercise cycling capacity when compared with a normal mixed diet (Greenhaff, Gleeson and

Maughan 1988a,b). Diet adherence was checked verbally prior to each trial. Furthermore, participants were screened to ensure that they were not currently undertaking or had undertaken a nutritional regime involving any alkalotic buffers such as $NaHCO_3$, NaCit or β-alanine within the previous 3-6 months. This information was included in the participant information sheet provided and confirmed verbally before participants gave written informed consent. Each participant also completed a general health screening questionnaire (GHQ) before each trial. Participants reported for each trial two to three hours postprandial after the same pre-exercise meal and at the same time of day to avoid any circadian rhythm effects on performance (Reilly 1990).

3.3 Equipment

3.3.1 Height and body mass

Height was measured using a stadiometer (Model 220, Seca, Hamburg, Germany) and body mass measured by digital floor standing scales (Model 770, Seca, Hamburg, Germany).

3.3.2 Cycle ergometry

The mode of exercise undertaken in all *in vivo* (i.e. human) studies (chapters 4, 5 and 7) was cycle ergometry (Monark 824E Ergomedic, Monark, Varberg, Sweden). The ergometer was calibrated by adding 4 Kg to the cradle and measuring the distance between the flywheel and the cradle during manual movement of the flywheel. The manufacturer's handbook recommended a distance of between 30 to 80 mm and for this setup the distance was typically ~ 55mm. Before commencing the initial peak oxygen uptake test, participants (in conjunction with the researcher) selected the seat and pedal strap positions that they felt most comfortable with ensuring the leg was slightly flexed when the feet reached the bottom of each rotation. These positions were adopted for all subsequent trials in that study. This

process was repeated for any participants who volunteered in more than one study to ensure consistency of process between participants.

3.3.3 Expired gas collection and analysis

Expired gas was sampled and analysed using an online breath-by-breath system (Metamax 3B, Cortex Biophysik, Leipzig, Germany) as per manufacturer guidelines. Before every test, this system was calibrated with gas concentrations (5% CO_2 and 15% O_2, British Oxygen Company, Surrey, UK) using a 6L antistatic re-breathable bag (Harvard Apparatus Ltd, Kent, UK), flow rate using a 3L calibration syringe (Hans Rudolf Inc, Kansas, USA) and atmospheric pressure using a manual wall mounted mercury barometer (F.Dalton & Co Ltd, Watford, UK). A face mask was secured over the participant's mouth and nose using a mesh harness covering the top of the head ensuring the mouthpiece was securely attached. Unless otherwise specified, breath-by-breath data were averaged over the last sixty seconds of the pre-exercise rest period (baseline) and for the last ten seconds of an exercise bout. Participants were blinded to the clock during rest to minimise any anticipatory changes in baseline physiology. Key data analysed calculated, oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), minute ventilation ($\dot{V}_E$) and respiratory exchange ratio (RER).

3.3.4 Heart rate

Heart rate (HR) was measured using a telemetric HR monitor (Polar FS1, Kempele, Finland) which recorded HR as a 'one-off' reading in real time. Baseline HR data was taken at the end of seated rest periods and remaining HR data was collected at pre-selected intervals during and post-exercise (see 3.6.1 and 3.6.2).

3.4 Perceptual variables

3.4.1. Ratings of perceived exertion

Ratings of perceived exertion (RPE) were measured using the Borg Scale (6-20; Borg, 1982). During familiarisation and experimental trials, RPE was collected for both overall and local fatigue. Local fatigue (RPE_L) was defined as exertion specific to the leg musculature and overall fatigue (RPE_O) was defined as a more general cardiovascular perception of fatigue (Robertson et al. 1986, Swank and Robertson 1989). Only RPE_O was collected for the graded incremental test. For continuous work trials to volitional exhaustion (T_{LIM}), RPE were recorded post-exercise and after 1 min (chapter 4), 1 and 2 mins (chapter 5) or 1, 2 and 3 mins (chapter 7) of exercise. Previous research in well trained males has demonstrated that measuring RPE in the first two minutes of short-term high intensity exercise is reliable (Doherty et al. 2001).

3.4.2. Abdominal discomfort and gut fullness

During experimental trials, ratings of abdominal discomfort (AD) and gut fullness (GF) were measured using an 11 point Likert scale (0-10; sections 10.1 and 10.2). Similar scales have been used in previous research using $NaHCO_3$ supplementation (Price, Moss, and Rance 2003, Price and Cripps 2012). Data were sought pre $NaHCO_3$ ingestion, 30 mins post ingestion, 60 mins post ingestion (pre-exercise) and immediately post-exercise.

3.5 Blood variables

3.5.1 Blood lactate

Blood was collected by means of finger-prick capillary samples. The finger was wiped with an isopropyl alcohol swab (Medlock Medical, Oldham, UK) and then punctured using a 1.8 mm lancing device (Safety Lancet, Sarstedt, Germany). The initial blood was wiped away using a tissue and the subsequent 20 µL was collected in a sodium heparinised capillary tube (EKF Diagnostic, Magdeburg, Germany). This was then added to a 1mL Eppendorf tube (EKF Diagnostic, Magdeburg, Germany) and mixed well. Samples were then

analysed for blood lactate concentration (Biosen C_line, EKF Diagnostic, Magdeburg, Germany).

3.5.1.i Reliability of Biosen C_line analyser

The test-retest reliability of the Biosen C_line analyser (EKF Diagnostic, Magdeburg, Germany) was evaluated in two ways. Firstly, using samples of known concentration supplied by the manufacturer (2.0, n=10; 7.0, n=10; 12.0, n=4; and 18.0, n=10) mmol.l^{-1}) and secondly, using blood samples collected at rest and after a range of high intensity exercise (n=29). Figures 3.1 and 3.2 highlight very high test-retest reliability for both methods, with R^2 values of 0.99, in line with previous analysis (Davison et al. 2000). Within-measurement CV of 1.2%, 0.4%, 0.1% and 0.5% were observed for the 2.0, 7.0, 12.0 and 18.0 mmol.l^{-1} standards, respectively. The mean within-measurement CV for physiological samples was 0.4%.

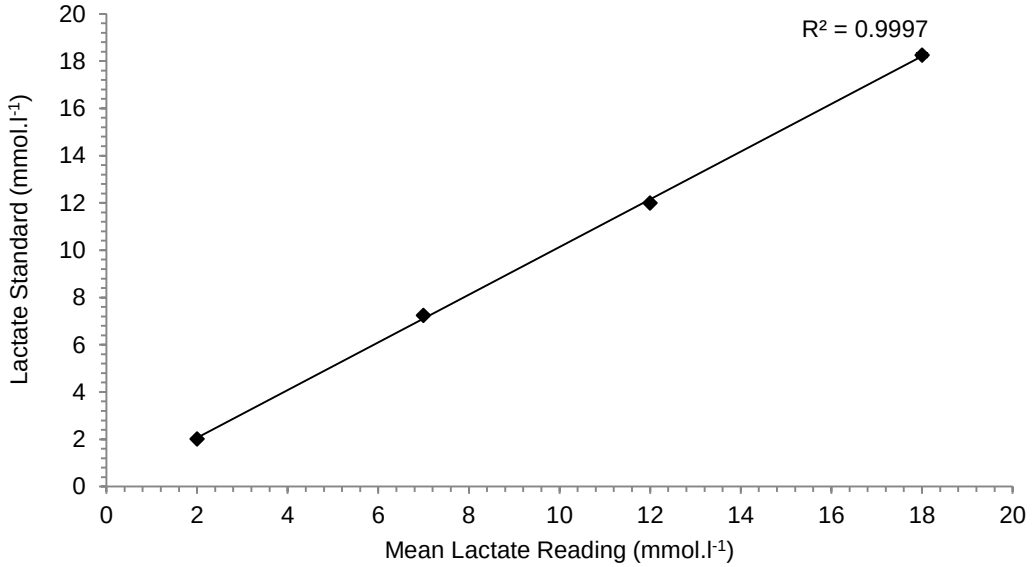

Figure 3.1 Biosen C_line test-retest reliability: Samples of known concentration

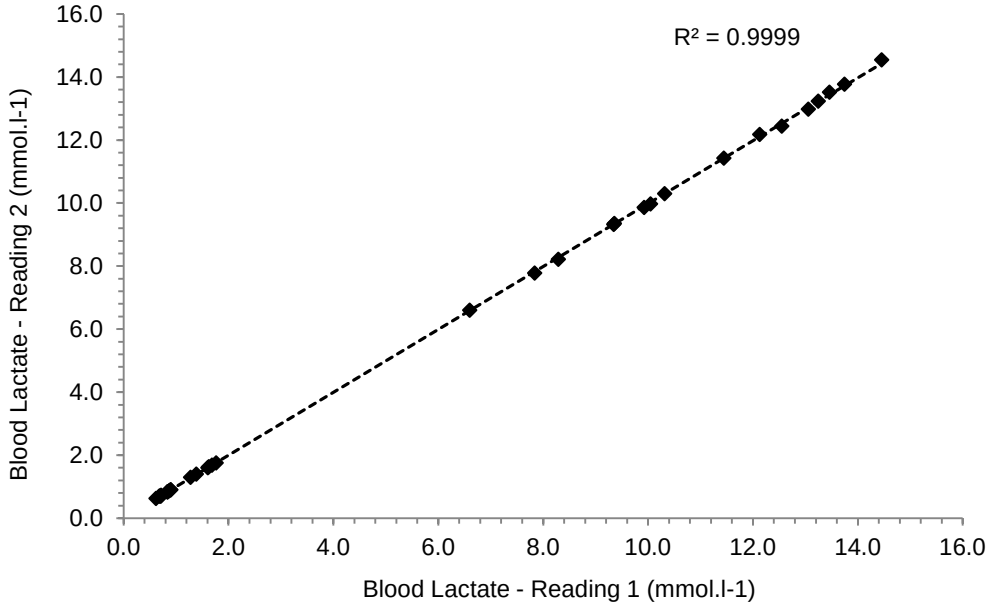

Figure 3.2 Biosen C_line test-retest reliability: Physiological samples

3.5.2 Base excess, pH and [HCO_3^-]

Base excess (BE), pH and bicarbonate ion concentration [HCO_3^-] were collected using finger-prick capillary samples. The same cleaning and lancing procedure was used as outlined in section 3.5.1 The sample was collected in a 100 µL clinitube (Radiometer Medical ApS, Copenhagen, Denmark), capped at both ends and mixed. Samples were then analysed using a blood gas analyser (ABL5 radiometer, Radiometer Medical ApS, Copenhagen, Denmark).

3.5.2.i Reliability of blood gas analyser (ABL5 Radiometer)

The test-retest reliability of the blood gas analyser (ABL5 radiometer, Radiometer Medical ApS, Copenhagen, Denmark) was completed by using samples of known concentration (7.12 (n=5), 7.15 (n=5), 7.38 (n=5), 7.62 (n=5); n=20). Figure 3.3 highlights very high test-retest reliability (R^2 = 0.99). Within-measurement CV of 0.1%, 0.2%, 0.2% and 0.1% were observed for the 7.12, 7.15, 7.38 and 7.62 standards, respectively.

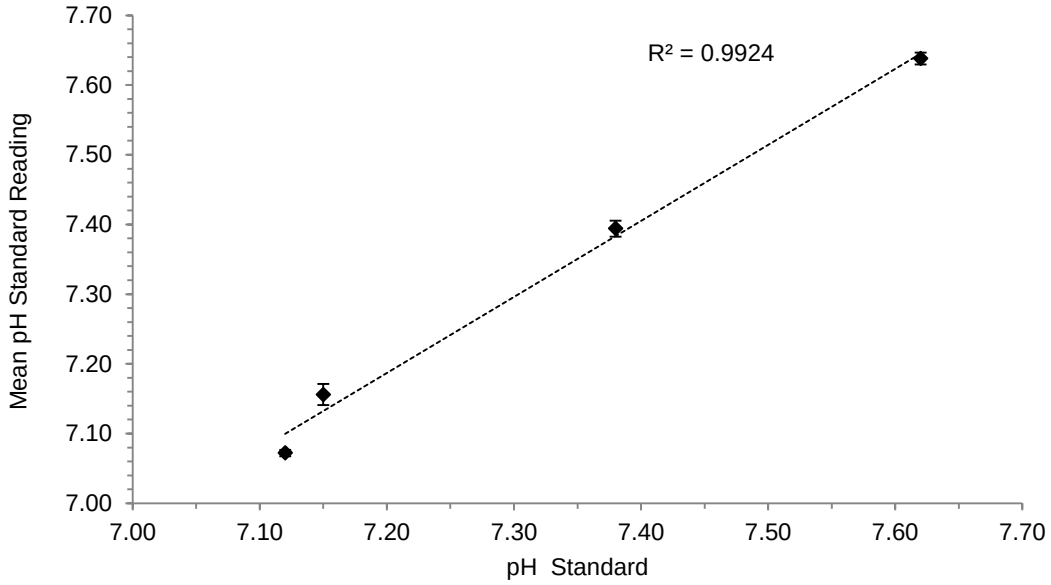

Figure 3.3 ABL5 radiometer test-retest reliability: Samples of known concentration

3.6 Exercise protocols

3.6.1 Graded incremental exercise test

A graded incremental exercise test to ascertain peak oxygen uptake ($\dot{V}O_{2PEAK}$) was completed by all participants prior to experimental trials. This test also identified the average peak minute power (W_{PEAK}) achieved by participants which was used to calculate the relative power output for subsequent exercise bouts. After gaining informed consent and completing the general health screening questionnaire (GHQ), both height and body mass were measured. Participants were then seated and rested quietly for five minutes with baseline ventilatory data analysed over the final minute (section 3.3.3). Heart rate was recorded (section 3.3.4) at the end of rest and baseline blood lactate (BLa) was taken before mounting the ergometer (section 3.5.1).

Cycling commenced on the ergometer (Monark 824E Ergomedic, Monark, Varberg, Sweden) at a cadence of 70 rev.min^{-1} with an unloaded cradle (70 W; 1 Kg). Participants continued pedalling at this cadence for three minutes. During the last five seconds of each

stage, HR and RPE_O were recorded. Upon completion of each stage 0.5 kg (35 W) load was added to the cradle. This process continued until volitional exhaustion with the researcher providing verbal feedback for maintenance of the specified cadence and to complete a maximal effort. Heart rate and RPE_O were recorded at volitional exhaustion and further BLa samples were taken immediately and five minutes post-exercise. W_{PEAK} was calculated as the mean power achieved during the final minute of the test (Lamberts et al. 2012). If exercise ceased across stages, the appropriate duration undertaken at each power output was used to calculate a pro-rata W_{PEAK}. For example, if a participant stopped exercising 40 seconds into stage 6, the following calculation was undertaken. W_{PEAK} = (20 s / 60 s) * 210 W (stage 5) + (40 s / 60 s) * 245 W (stage 6) = (70 + 163) = 233 W.

3.6.1.i Reliability

Eight healthy and active, but none specifically trained males completed two incremental exercise tests to volitional exhaustion to ascertain their peak oxygen uptake and associated W_{PEAK}. The same pre-exercise screening process was carried out as described in section 3.2 and trials were conducted 3-7 days apart. The procedure for the exercise test followed the same protocol as outlined in section 3.6.1.

Mean age, body mass and height were 21.4 ± 4.8 years, 85.2 ± 13 Kg and 179.4 ± 5.6 cm, respectively. The data collected from both tests are presented in table 3.1. Unfortunately, due to equipment error, respiratory data was unable to be collected for n=3 on test 2 and thus due to unequal groups (n=8 vs. n=5) a Mann-Whitney U test was used to analyse this data. RPE was analysed using a Wilcoxon test due to non normality of data. For the remaining variables paired t-tests were used. The appropriate parametric (Pearson; r) or non-parametric correlations (Spearman's Rho; ρ) were also applied where appropriate.

Table 3.1 Physiological data from repeat graded incremental tests

	Physiological Data					Correlation Data		
Variable	**Test 1**	**Test 2**	**± SE**	**P**	**CV_{WS}**	**r**	**r^2**	**P**
Performance time (s)	1043 ± 104	1045 ± 86	2 ± 27	0.88	2.1%	0.93	0.87	0.001
W_{PEAK} (Watts)	255 ± 24	253 ± 18	-2 ± 11	0.71	2.9%	0.75	0.57	0.03
$\dot{V}O_2$ ($l.min^{-1}$) **	3.59 ± 0.6	3.48 ± 0.8 **	-0.1 ± 0.4	0.83 *	8.0%	0.87	0.76	0.05
$\dot{V}O_2$ ($ml.kg^{-1}.min^{-1}$) **	42 ± 13	42 ± 16 **	-0.8 ± 4.5	0.62 *	8.3%	0.82	0.67	0.09
$\dot{V}_E$ ($l.min^{-1}$) **	111.4 ± 12.6	113.9 ± 9.3 **	-2.5 ± 7.0	0.52 *	5.0%	0.46	0.21	0.43
End-exercise HR (bpm^{-1})	195 ± 7	192 ± 7	-3 ± 3	0.10	1.2%	0.87	0.76	0.01
End-exercise RER **	1.01 ± 0.15	0.96 ± 0.06 **	0.01 ± 0.04	0.72 *	3.9%	0.56	0.31	0.32
End-exercise BLa ($mmol.l^{-1}$)	12.2 ± 1.8	11.9 ± 2.2	-0.1 ± 1.2	0.93	8.4%	0.65	0.42	0.08
5 mins post-exercise BLa ($mmol.l^{-1}$)	11.2 ± 1.9	10.8 ± 2.4	-0.4 ± 1.0	0.52	8.7%	0.79	0.63	0.02
RPE_O	20 ± 1	20 ± 0	0.1 ± 0.3	0.50	0.4%	0.75	0.56	0.03

Note: * P based on n=8 vs. n=5 (Mann Whitney U test) ** n=5 (including correlation and CV_{WS} data)

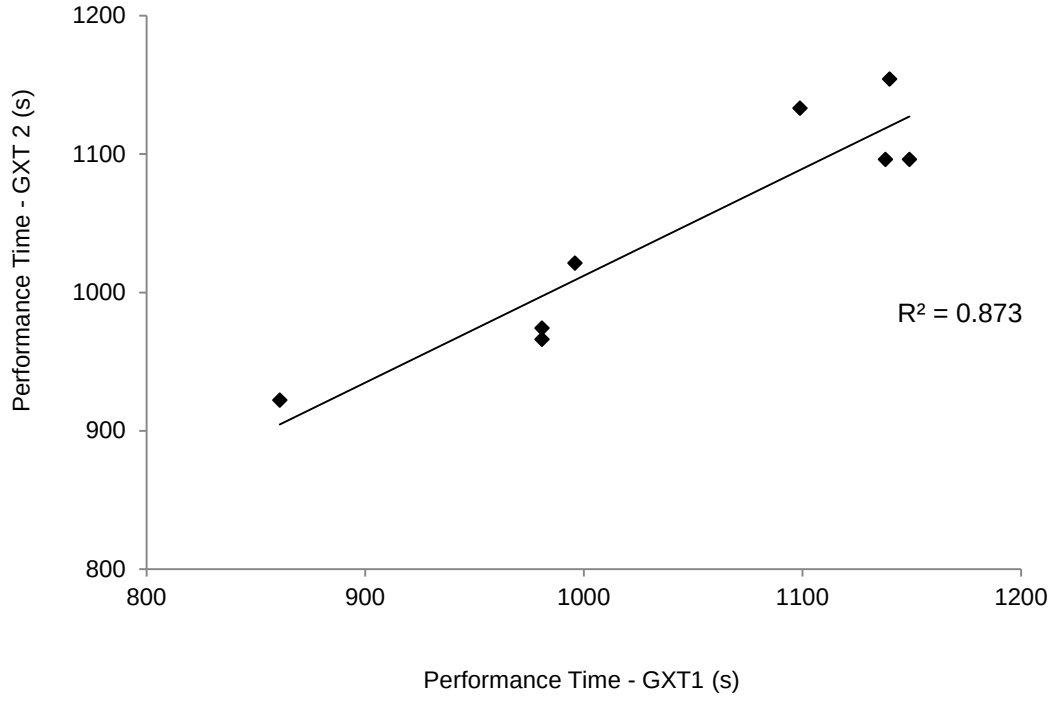

Figure 3.4 Correlation between performance times for repeated graded incremental exercise tests

There were no significant differences between incremental test 1 and 2 for any of the variables measured (Table 3.1). There were significant correlations for performance time (Figure 3.4), $W_{PEAK,}$ $\dot{V}O_2$ ($l.min^{-1}$), end-exercise HR (Figure 3.5), 5 mins post-exercise BLa and RPE_O. The correlations for $\dot{V}O_2$ ($ml.kg^{-1}.min^{-1}$) and post-exercise BLa approached significance (Table 3.1).

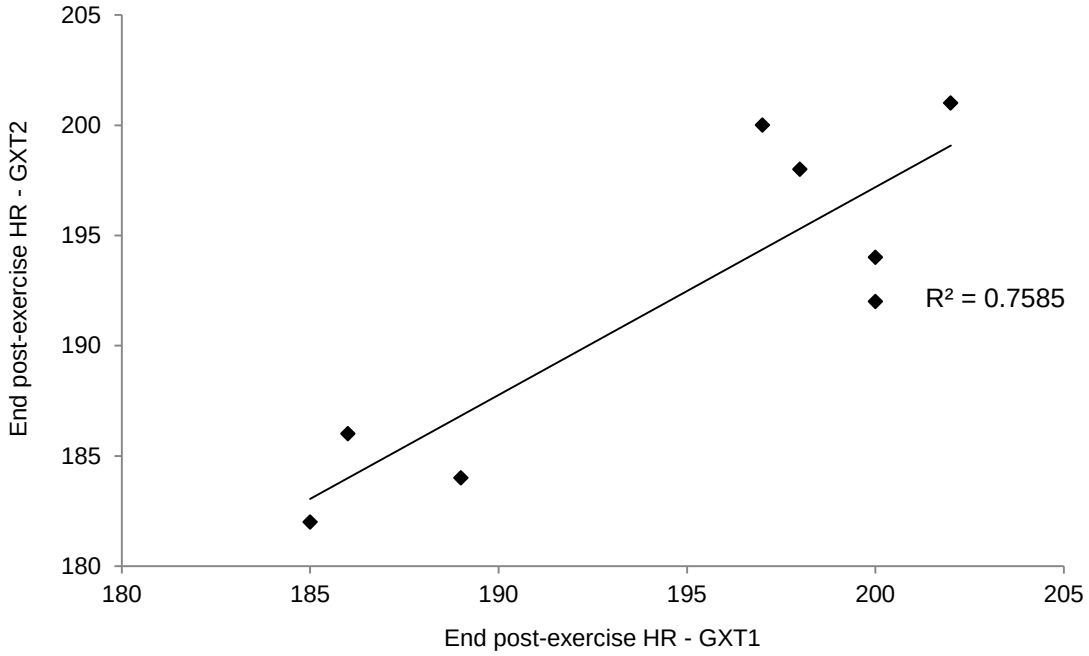

Figure 3.5 Correlation between end post-exercise heart rate (HR) for repeated graded incremental exercise tests

Table 3.2 demonstrates the magnitude based inferences that can be made between tests as described by Hopkins et al. (2009). More simply, an indication as to the probability of each possible outcome (i.e. T1 > T2, T1 = T2 or T1 < T2) is presented based on the calculated test statistic, P value and the smallest worthwhile change (SWC). Due to the non-elite status of participants, SWC for each variable was calculated as 0.2 * the between-subject grouped standard deviation of the raw scores (Hopkins 2000). A standardised change in mean (i.e. effect size; ES) was also calculated as per section 3.8. Only variables that were normally distributed and where n=8 have been calculated using this method (See

Table 3.2). With the exception of post-exercise HR there is ≥ 85% likelihood of negligible difference between tests 1 and 2 in all variables (Table 3.2) with effect sizes lower than the smallest worthwhile change of 0.2 * between-subject SD. Moreover, a difference of 3 bpm^{-1} for post-exercise HR between test 1 and 2 is more likely to represent daily biological variation and hence unlikely to be physiologically different (Table 3.1, Figure 3.5).

Combined with the correlation data and traditional statistical analysis previously presented (Table 3.1) we conclude that one graded incremental test is sufficient to ascertain $\dot{V}O_{2PEAK}$ / W_{PEAK} for use in subsequent experimental trials. These results are supported by previous data examining $\dot{V}O_{2MAX}$ during successive maximal cycle ergometer tests in a similar participant cohort to that used in the present study (Foster et al. 2007).

Table 3.2 Magnitude based inferences for repeated incremental test data

Variable	SWC	T2 >T1	T1 = T2	T2 < T1	ES
Performance time	1.8%	7.0%	88.5%	4.4%	0.02
W_{PEAK}	1.7%	3.8%	84.6%	11.7%	0.10
End-exercise HR	0.7%	1.7%	11.2%	87.1%	0.35
End-exercise BLa	3.3%	0.6%	98.7%	0.7%	0.14
5 mins post-exercise BLa	4.0%	0.1%	99.2%	0.6%	0.16

Note: SWC = smallest worthwhile change, 0.2 * B-S SD (%), ES = Effect Size (standardised mean difference)

3.6.1.ii Validity

Bird and Davison (1997) outline a number of criteria that require consideration in establishing maximal oxygen uptake ($\dot{V}O_{2MAX}$) in adults. They are: (1) final HR within 10 bpm^{-1} of the age-related maximum (we calculated this as 208 – (0.7 * age); Tanaka, Monahan, and Seals 2001), (2) ~ 5 min post-exercise BLa of 8 $mmol.l^{-1}$ or greater, (3) subjective fatigue and volitional exhaustion, (4) RPE rating of 19-20 on the Borg Scale or equivalent

and (5) RER ≥ 1.15. It is also stated that a plateau in oxygen uptake, defined as an increase in oxygen uptake of less than 2 $ml.kg^{-1}$ or 3% with an increase in exercise intensity should also be considered. However, there is no universally accepted definition of the $\dot{V}O_2$ plateau (Astorino, White, and Dalleck 2009). Furthermore, a 'true' $\dot{V}O_{2MAX}$ may not be achieved due to local muscular fatigue when using an unfamiliar mode of exercise such as a cycle ergometer in none cycling specifically trained participants (Astorino, White, and Dalleck 2009). In the absence of such a plateau, and where other parameters have been met, participants are deemed to have reached their $\dot{V}O_{2PEAK}$.

With the exception of a post-exercise RER of ≥ 1.15 participants met all criteria as outlined by Bird and Davison (1997). In parallel with visual inspection of each participants $\dot{V}O_2$ data, we conclude that participants reached $\dot{V}O_{2PEAK}$ rather than $\dot{V}O_{2MAX.}$ As such the graded incremental protocol utilised within this thesis is sufficient to ascertain participants' $\dot{V}O_{2PEAK}$ and W_{PEAK} during one exercise bout.

3.6.2 Time to volitional exhaustion (T_{LIM})

For all human studies a time to volitional exhaustion (T_{LIM}) protocol was adopted. This involved participants cycling at a pre-determined power output (calculated from initial incremental test, see section 3.6.1) until they were unable to maintain that power output for a specific period of time (e.g. ~ 3 to 4 seconds) or they stopped exercising completely. This protocol was chosen due to its wide adoption in the evaluation of ergogenic aids (Hill et al. 2007, Sale et al, 2011, Saunders, et al. 2011). Moreover, using a T_{LIM} protocol appears the most likely to demonstrate ergogenic benefit for $NaHCO_3$ supplementation (Matson and Tran 1993).

Upon completion of baseline data collection, the participant mounted the ergometer and commenced a warm up consisting of cycling at 70 $rev.min^{-1}$ for 4 min at 50% W_{PEAK}, 1

min at 75% W_{PEAK} and then 2 min at 70 W. After a verbal countdown the test commenced with participants blinded to the clock throughout. The cadence of 70 rev.min^{-1} was chosen as research examining a range of power outputs (100 – 300 W) and cycling cadences (30 – 120 rev.min^{-1}) during constant load cycling found 70 rev.min^{-1} to be optimal from both metabolic and respiratory perspectives (Ansley and Cangley 2009). Moreover, this cadence has been adopted by research examining the efficacy of $NaHCO_3$ ingestion on 100% W_{PEAK} cycling performance (Linderman et al. 1992).

A stationary start was employed which has previously been used in evaluating high-intensity cycling in a laboratory setting with active but not specifically cycling trained males, similar to the present study (Wittekind, Micklewright, and Beneke, 2011). The location of the desktop online breath-by-breath system (lab bench adjacent to cycle ergometer) meant that small adjustments in participant posture were possible but not sufficient space was afforded to substantially affect participant performance. The test was ceased the second time the cadence dropped below 70 rev.min^{-1} for more than 3 or 4 seconds or if the participant was unable to re-establish the required cadence within 3 to 4 seconds. Upon completion of the test, the participant was encouraged to cycle for 5 minutes at 70 W (unloaded cradle) to warm down and avoid syncope.

3.7 Treatment

For experimental studies 2 (chapter 5) and 4 (chapter 7) treatments were either 0.3 g.kg^{-1} body mass $NaHCO_3$ (McNaughton 1992a) or 0.1 g.kg^{-1} body mass NaCl (PLA). Treatments were administered single blind as per similar research (Price, Moss, and Rance 2003). Treatment solutions constituted of 4 ml.kg^{-1} water and 1 ml.kg^{-1} of double strength no added sugar orange squash (Sainsbury's, London, UK). In contrast to previous research pilot testing suggested that 0.3 g.kg^{-1} body mass $NaHCO_3$ and an equimolar dosage of NaCl

(0.21 g.kg^{-1} body mass) were unable to be matched for taste. Therefore, a dosage of 0.1 g.kg^{-1} of NaCl was used to match taste as closely as possible. All solutions were refrigerated overnight before consumption to enhance palatability (Price and Singh 2008).

3.8 Statistical analysis

Statistical analysis was completed using PASW (SPSS; v17, Chicago, USA). For all analyses, standard normality and homogeneity of variance/sphericity (Shapiro-Wilk and Mauchly tests respectively) were checked prior to using parametric tests (i.e. t-tests, 1, 2 or 3-way repeated measures ANOVA). For interactions, Tukeys' post hoc analysis was undertaken by calculating the difference required between means for significance at the level of $P < 0.05$ (Vincent 1999). For any violations of sphericity, degrees of freedom were corrected using Huynh-Feldt ($\varepsilon > 0.75$) or Greenhouse-Geiser ($\varepsilon < 0.75$) values for ε, where applicable (Field 2005). Where normality of data was not confirmed, non parametric tests were used (i.e. Wilcoxon, Mann Whitney U or Friedman). Traditional statistical significance was accepted at $P < 0.05$ although, where appropriate, exact P values are presented. The within-subject coefficient of variation (CV_{WS}) was calculated as the mean within-subject SD / mean variable scores (Hopkins, Schabort and Hawley 2001). Other figures quoted are mean values ± standard deviation unless otherwise stated.

3.8.1 Magnitude based inferences

In addition to the traditional statistical analysis, magnitude based inferences, as described by Batterham and Hopkins (2006) and Hopkins et al. (2009) are presented where appropriate. Using the calculated test statistic, P value and the smallest worthwhile change (SWC) a specialist spreadsheet (Hopkins 2007) was used to calculate the probabilities of whether a specific outcome is (1) positive / beneficial, (2) trivial / negligible or (3) negative / harmful. The SWC for studies 1 and 2 (chapters 4 and 5) was calculated as 0.2 * between-

subject SD of peak power from the baseline incremental test. For study 4 (chapter 7) the SWC was calculated for both pre and post-training incremental tests and used to calculate probability of changes based on pre and post-training T_{LIM} performance, respectively. Calculation of SWC using this approach was suggested by both Professors Will Hopkins and Alan Batterham (2012, personal communications). A standardised change between trials was calculated as the effect size (ES = change in mean / pooled between-subject SD) and compared, where appropriate, against the smallest worthwhile change of 0.2 * between-subject SD (Hopkins 2004). Where appropriate odds ratios are presented the recommended benefit: harm threshold is > 66. More simply, this means that for the given variable there is > 25% chance of benefit and < 0.5% chance of harm (Hopkins 2007).

3.8.2 Missing data

Unfortunately, due to operator/equipment error, a small amount of experimental data could not be recorded (0.7% of total). To avoid deleting otherwise complete datasets (i.e. decreased power of statistical analysis), these values were calculated using an adapted version of hot deck imputation (HDI; Schafer and Graham 2002). Hot deck imputation uses data from other observations within the data set to estimate missing values. Where possible, data from other tests from the same participant was used. The percentage of data estimated using this method from each study is detailed below along with specific examples. This unobtainable data is confined to blood pH and HR data only from experimental trials only. No performance time (T_{LIM}), BLa, respiratory or perceptual data or data from any incremental test or the isolated muscle study (chapter 6) was estimated using this method.

- Study 1 (chapter 4) – HDI Data = 0.8% (5 / 638)

- Resting pH for trial two (FAM2) for participant 1 (completed study 1 and 2) was estimated as the mean of their resting pH for FAM1 and FAM3 and the six experimental sessions (EXP01-EXP06)

- Post-exercise HR for FAM1, participant 11 was estimated as resting HR trial 1 plus the mean difference between pre and post-exercise HR for the other 32 trials

- Study 2 (chapter 5) – HDI Data = 0.2% (5 / 2520)

- 5 mins post-exercise HR for the 100% W_{PEAK} PLA and 120% W_{PEAK} $NaHCO_3$ trials, for participant 1 was estimated as the mean post-exercise HR from all other experimental trials (EXP02-EXP05)

- Study 4 (chapter 7) – HDI Data = 1.5% (27 / 1800). The reason that this figure is relatively higher than previous studies relates to the fact that pH, BE and $[HCO_3^-]$ are collected as one sample which equates to three pieces of data per unobtainable sample

- Pre $NaHCO_3$ ingestion values for pH, BE and $[HCO_3^-]$ for participant 5 in the post-training PLA trial were calculated as the mean pH, BE and $[HCO_3^-]$, from the pre-ingestion and pre-exercise values from the pre-training PLA trial and the pre-exercise values from the post-training PLA trial

- 5 mins post-exercise pH, BE and $[HCO_3^-]$ values for participant 2 in the pre-training PLA trial were calculated as post-exercise value plus mean difference of post and 5 mins post-exercise values for all other participants

Chapter 4 - Familiarisation to and reproducibility of cycling at 110% peak power output.

4.1 Abstract

This study investigated the familiarisation to and test re-test reproducibility of constant load cycling at 110% peak power output (W_{PEAK}). Eleven healthy, but not cycle trained, males performed a graded incremental exercise test to ascertain W_{PEAK} followed by three trials (T1, T2 and T3) at 110% W_{PEAK} to exhaustion. Trials were separated by ~ 7 days. Although there was no difference in time to exhaustion (T_{LIM}) between T1 and T2 (P = 0.100) and T2 and T3 (P = 0.095) respectively, a difference was observed between T1 and T3 (P = 0.046). Correlation coefficients, coefficients of determination, limits of agreement (LoA) and within-subject coefficient of variation (CV_{WS}) improved across trials demonstrating T2 and T3 had the strongest relationship (T1 vs. T3: r = 0.73; r^2 = 0.53; Bias = 40 s; CV_{WS} = 14%; T1 vs. T2: r = 0.66; r^2 = 0.43; Bias = 24 s; CV_{WS} = 10%; T2 vs. T3: r = 0.97; r^2 = 0.95; Bias = 16 s; CV_{WS} = 7%). There was no difference across trials for cardiorespiratory (HR, RER, $\dot{V}_E$, $\dot{V}O_{2,}$), blood (BLa, pH) or perceptual variables (RPE_L, RPE_O). In conclusion, constant load cycling at 110% W_{PEAK} is a reliable protocol when assessing supramaximal exercise capacity after completion of two familiarisation trials.

4.2 Introduction

In order to be of optimal value a performance test must be reliable, valid and sensitive (Mendez-Villaneuva, Bishop, and Hamer 2007, Currell and Jeukendrup 2008). However, no research on the validity of physical performance measures has included reliability of the criterion measure(s) (Hopkins, Schabort, and Hawley 2001). Until such work is completed Hopkins, Schabort, and Hawley (2001) suggest that tests of high reliability should be used as only these tests can have high validity. Reliability of performance testing

refers to the consistency of performance on repeated tests with greater reliability resulting in more precise performance data (Hopkins 2000, Hopkins, Schabort, and Hawley 2001, Watt, Hopkins, and Snow 2002). The main components of reliability are systematic bias such as learning effects or fatigue and random error due to biological or mechanical variation (Atkinson and Nevill 1998). To minimise systematic bias participants are often familiarised with the proposed tests before collecting performance data (Carey and Richardson 2003). By performing enough familiarisation trials, learning effects or other systematic changes are diminished sufficiently so that reliable performance data can be obtained (Hopkins 2000). Indeed, there is clear evidence of a learning effect between the first two trials of anaerobic performance tests and thus a minimum of one familiarisation trial before experimental testing is required (Hopkins, Schabort, and Hawley 2001, Barfield et al. 2002). Research evaluating the reliability of singular or repeated high-intensity cycling sprints (Capriotti, Sherman, and Lamb 1999, Glaister et al. 2003, Mendez-Villaneuva, Bishop, and Hamer 2007) found that high reliability is achieved after two familiarisation trials. This research regarded high reliability as exhibiting a CV_{WS} of ~ 2-4%.

Hopkins, Schabort, and Hawley (2001) analysed the reliability of physical performance tests by converting the performance criteria from constant power time to exhaustion tests (T_{LIM}), into an estimate of mean power output and comparing its reliability against a range of other physical performance tests. The authors reported that although the CV is greater for constant power tests (~ 16%) this is because a small change in power output will result in a much larger change in time to exhaustion (T_{LIM}). Indeed, the authors suggest that constant power tests are the most reliable physical performance test (Hopkins, Schabort, and Hawley 2001).

The generation of supramaximal power during brief ‘all-out’ exercise is an important facet of many sports (Mendez-Villaneuva, Bishop, and Hamer 2007). A number of tests, such as the Wingate Anaerobic cycling Test (WAnT; Ayalon, Inbar, and Bar-Or 1974, Inbar,

Bar-Or, and Skinner 1996) and the 6 s maximal cycling sprint test (Mendez-Villaneuva, Bishop, and Hamer 2007) have been developed to provide such measures of anaerobic performance. However, both the 6 s maximal cycling sprint tests and 30 s WAnT tests are focussed almost exclusively on anaerobic energy provision and are therefore most suitable for measuring exercise capacity up to ~ 30 seconds. Other performance measures, such as constant load exercise to volitional exhaustion (e.g. 110% of W_{PEAK}) or 'all-out' exercise for longer than 60 seconds, incorporates a greater contribution of ATP from aerobic metabolism (Gastin et al. 1995). Studies that have examined the physiological responses to high intensity workloads lasting between 60 and 180 seconds have either utilised constant duration tests (Carey and Richardson 2003, Carter et al. 2005, Burnley, Doust, and Vanhatalo 2006) or a combination of constant load and constant duration cycling (Doherty et. al. 2003) and thus have not examined time to volitional exhaustion as the outcome measure. Studies adopting cycling at 110% $\dot{V}O_{2PEAK}$ have either compared performance time against other exercise intensities (Gastin et al. 1995), assessed maximal accumulated oxygen deficit (MAOD; Weber and Schneider 2001, Bertuzzi et al. 2010) or evaluated ergogenic aids (Hill et al. 2007, Sale et al. 2011, Saunders et al. 2011). At the time of starting this study there was no reported research which sufficiently assessed the reliability of T_{LIM} at 110% $\dot{V}O_{2PEAK}$. It is acknowledged that Saunders et al. (2012) have published a similar study in the interim period and analysis of this research will be incorporated into section 4.5 (Discussion).

The ability to detect changes outside of day-to-day variation in exercise performance / capacity (i.e. sensitivity) is of particular importance in research where an intervention such as nutritional supplementation is evaluated (Hill et al. 2007, Sewell and McGregor 2008, Sale et al. 2011, Saunders et al. 2011, O'Hara et al. 2012). Despite assertions that time-trials may be more sensitive in detecting changes in endurance capacity, constant load tests are at least as sensitive (Amann, Hopkins, and Marcora 2008). Although such measures have been investigated in trained cyclists there is little research in those with little experience

of laboratory testing (Sewell and McGregor 2008). This lack of research exists despite the large amount of studies in which non-specifically trained but recreationally active individuals form the experimental cohort and indeed the large amount of nutritional products purchased and consumed by this population. Therefore, the aim of this study was to investigate the familiarisation to and test re-test reliability of continuous supramaximal cycling at 110% peak power output (W_{PEAK}) in recreationally active male participants not well accustomed to cycling. We hypothesised that two trials would be required before participants become fully familiarised and reliable data was obtained.

4.3 Methods

4.3.1 Participants

Eleven healthy males volunteered to take part in this study (mean ± SD: age 23.6 ± 3.7 years, body mass 74.3 ± 10.7 kg, height 175 ± 4 cm, peak oxygen uptake ($\dot{V}O_{2PEAK}$) 41.0 ± 6.2 $ml.kg^{-1}.min^{-1}$) which had received University Ethics Committee approval. All participants were recreationally active undertaking 2-3 exercise sessions (e.g. football, rugby, and/or running) per week. None were specifically cycling trained.

4.3.2 Pre-experimental procedures

Participants were requested to avoid alcohol and strenuous exercise for at least 24 hours prior to exercise, to maintain the same balanced mixed diet and to avoid caffeine for at least 12 hours before tests. Adherence to these pre-test guidelines was checked verbally prior to each trial. All participants gave written informed consent and completed a general health screening questionnaire before each bout. Participants reported for each trial two hours postprandial and at the same time of day to avoid any circadian rhythm effects on performance (Reilly 1990). Trials were carried out seven days apart.

4.3.3 Study design

Participants visited the laboratory on four separate occasions. Trials were separated by ~ 7 days. Each participant performed a graded incremental exercise test to ascertain peak power output (W_{PEAK}) followed by three exercise trials (T1, T2 and T3) at 110% W_{PEAK} to exhaustion (T_{LIM}). We adopted T_{LIM} as criteria for exercise capacity because of its wide adoption in evaluating ergogenic aids (Hill et al. 2007, Sale et al, 2011, Saunders, et al. 2011). The intensity of 110% W_{PEAK} was chosen as this intensity appears with some frequency within the sports science literature (Gastin et al. 1995, Weber and Schneider 2001, Hill et al. 2007, Bertuzzi et al. 2010, Sale et al, 2011, Saunders, et al. 2011) but at the time of commencing this research the associated reliability had not been reported. Furthermore, it has been acknowledged that evaluation of protocols in non trained participants is lacking (Sewell and McGregor 2008).

4.3.4 Incremental exercise test

On the first visit to the laboratory, participants completed a graded incremental exercise test on a cycle ergometer (Monark 824E Ergomedic, Monark, Varberg, Sweden) to ascertain $\dot{V}O_{2PEAK}$ and W_{PEAK}. Before commencing the exercise test, each participant selected the seat and pedal strap positions that felt most comfortable ensuring that the leg was slightly flexed when the feet reached the bottom of each duty cycle. These settings were adopted for all subsequent trials. The full incremental exercise test protocol can be found in section 3.6.1.

4.3.5 Experimental trials

On the three subsequent visits to the laboratory, participants cycled to volitional exhaustion (T_{LIM}) at a relative power output of 110% W_{PEAK}. The cadence of 70 rev.min^{-1} was

chosen as research examining a range of power outputs (100 – 300 W) and cycling cadences (30 – 120 rev.min^{-1}) during constant load cycling found 70 rev.min^{-1} to be optimal from both metabolic and respiratory perspectives (Ansley and Cangley 2009). As the participants in the present study were not well trained cyclists and the 110% W_{PEAK} test uses ~ 80% aerobic energy (Gastin et al. 1995) the cadence of 70 rev.min^{-1} would likely optimise T_{LIM} and thus enhance familiarisation to the test.

Once breathing in to the online breath-by-breath system participants sat quietly for fifteen minutes. On completion of the rest period, HR was noted and blood samples were taken for measurement of blood lactate concentration (BLa) and pH. Cardiorespiratory and blood data were collected as per sections 3.3.3, 3.3.4 and 3.5.1, 3.5.2 respectively. On completion of the rest period the participant mounted the ergometer and commenced a warm up consisting of; cycling at 70 rev.min^{-1} for four minutes at 50% W_{PEAK} (general cardiovascular stage), one minute at 75% W_{PEAK} (medium intensity stage), and then two minutes at 70 W (unloaded cradle; maintenance stage). By using a variety of exercise intensities this protocol was designed to ensure participants were adequately warmed up prior to each trial. After a verbal countdown the test commenced with participants blinded to the clock. Stationary starts have been successfully been used in evaluating high-intensity cycling performance in a laboratory setting with active but not specifically cycle trained males (Wittekind, Micklewright, and Beneke 2011) similar to the subject cohort in the present study. All participants achieved the required cadence within ~ 10 to 15 seconds. After any initial drop in cadence the researcher verbally encouraged the participant to re-establish the desired cadence to ensure consistent power output was maintained. The test was ceased the second time the cadence dropped below 70 rev.min^{-1} for more than 3 or 4 seconds or if the participant was unable to re-establish the required cadence within 3 to 4 seconds as outlined in section 3.6.2. Local RPE (RPE_L) and overall RPE (RPE_O) were recorded after one minute and upon cessation of exercise (Robertson et al. 1986, Swank and Robertson 1989; section 3.4.1). Capillary blood samples were taken immediately on volitional

exhaustion (BLa) and five minutes post-exercise (BLa, pH). Upon completion of the test, the participant was encouraged to cycle for five minutes to warm down and to avoid syncope.

4.3.6 Statistical analysis

Statistical analysis was completed using PASW (SPSS; v17, Chicago, USA). Statistical significance, normality and homogeneity of variance/sphericity of data was assessed / adjusted as outlined in section 3.8. Exercise capacity (T_{LIM}) and other variables were analysed by one-way repeated measures ANOVA and subsequent pairwise comparisons (least significant difference; LSD) with the exception of RPE_O which was analysed using a Friedman test due to non-normality of data. The difference between RPE_L and RPE_O for each trial was therefore evaluated using separate Wilcoxon tests. The LSD comparisons were chosen as they are the most powerful when analysing 3 levels/groups (Maxwell and Delaney 2004, Cardinal and Aitken 2006, Howell 2007).

Test re-test reliability was determined using a combination of Bland-Altman plots (bias, limits of agreement), Pearson correlation coefficients (r), coefficient of determination (r^2) and within-subject coefficient of variation (CV_{WS}). The CV_{WS} was calculated as the mean within-subject SD / mean variable scores (Hopkins, Schabort, and Hawley 2001). Figures quoted are mean values ± standard deviation unless otherwise stated. Confidence intervals (95%CI) which define the range for which the true value of the statistic is 95% likely to fall (Watt, Hopkins and Snow 2002) were calculated as ± 1.96 * SE for individual trials, where SE = SD / √n (Streiner 1996) and for mean difference scores as ± 1.96 * SE for where SE = SD / √2 (Hopkins 2000, Hopkins, Schabort and Hawley 2001).

In addition to the traditional statistical analysis, magnitude based inferences, are presented where appropriate as outlined by section 3.8.1. For T_{LIM} the smallest worthwhile

change (%) was calculated as 0.2 * between-subject CV of W_{PEAK} derived from the baseline incremental exercise test (Hopkins 2000, Hopkins 2004).

4.4 Results

Mean $\dot{V}O_2$, $\dot{V}_E$, HR, RER, blood lactate concentration (BLa) and RPE_O values at the end of the initial peak oxygen uptake test exercise were 3.0 ± 0.5 l.min^{-1}, 114 ± 21 l.min^{-1}, 185 ± 12 bpm^{-1}, 1.05 ± 0.00, 10.3 ± 2.5 mmol.l^{-1} and 20 ± 0 respectively. This data supports the criteria for achievement of valid peak oxygen uptake tests (Bird and Davison 1997). Average W_{PEAK} in the final minute of the test was 225 ± 29 W and therefore mean 110% W_{PEAK} was 247 ± 32 W. A significant difference was observed for T_{LIM} across trials (P = 0.049; Figure 4.1, Table 4.1). Although there was no significant difference in T_{LIM} between T1 and T2 (LSD: P = 0.100) and T2 and T3 (LSD: P = 0.095) respectively, there was a significant difference between T1 and T3 (LSD: P = 0.046; Figure 4.1).

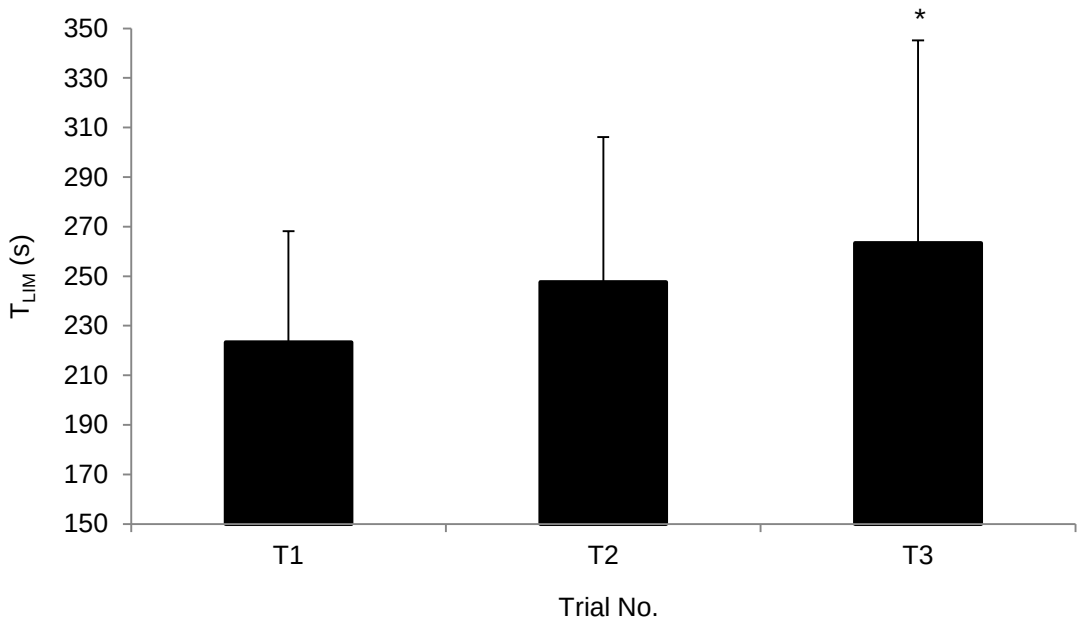

Figure 4.1 Time to volitional exhaustion (T_{LIM}) at 110% W_{PEAK} (n=11). * T 3 > T1; P < 0.05

There was no difference across trials for cardiorespiratory (HR, RER, $\dot{V}_E$, $\dot{V}O_2$), blood (BLa, pH) or perceptual variables (RPE_L, RPE_O; Table 4.1). A significant difference was

observed between mean RPE_L (20 ± 0) and RPE_O (19 ± 1) at the end of exercise (P = 0.017, P = 0.011 and P = 0.026 for T1, T2 and T3 respectively; Table 4.1). Correlation coefficients (r), coefficient of determination (r^2), limits of agreement (LoA) and improved across trials demonstrating T2 and T3 to have the strongest relationship (Figure 4.2; Table 4.2).

Table 4.1 Physiological data from each T_{LIM} trial

	Trial				
	T1	**T2**	**T3**	**Mean**	**P**
T_{LIM} (s) ± SD	223 ± 45	248 ± 59	263 ± 82 *	245 ± 64	0.049
95%CI (s)	197 – 250	213 – 282	215 – 312	207 – 282	N/A
$\dot{V}O_2$ ($l.min^{-1}$)	3.2 ± 0.7	3.2 ± 0.6	3.3 ± 0.7	3.2 ± 0.7	0.33
$\dot{V}O_2$ ($ml.kg^{-1}.min^{-1}$)	43 ± 7	44 ± 8	44 ± 8	44 ± 8	0.75
$\dot{V}_E$ ($l.min^{-1}$)	123 ± 23	121 ± 14	129 ± 23	124 ± 20	0.32
HR (bpm^{-1})	183 ± 11	185 ± 11	185 ± 10	184 ± 10	0.12
RER	1.12 ± 0.1	1.09 ± 0.1	1.10 ± 0.2	1.10 ± 0.1	0.52
BLa ($mmol.l^{-1}$)	11.9 ± 2.0	11.7 ± 2.0	12.3 ± 2.4	12.0 ± 2.1	0.76
5 mins post-ex BLa ($mmol.l^{-1}$)	11.6 ± 2.6	11.5 ± 2.3	11.7 ± 2.9	11.6 ± 2.5	0.76
5 mins post -exercise pH	7.24 ± 0.04	7.23 ± 0.05	7.23 ± 0.06	7.24 ± 0.05	0.47
RPE_O	19 ± 1	19 ± 1	19 ± 1	19 ± 1	0.91
RPE_L	20 ± 0	20 ± 0	20 ± 0	20 ± 0 #	1.00

Note: All values from end of exercise otherwise stated. * T3 > than T1 (P < 0.05) # RPE_L > than RPE_O (P < 0.05)

Magnitude based inferences for T_{LIM} (Table 4.3) demonstrated that T1 and T3 had a ~ 40% chance of substantial difference. Interestingly, data for T1 and T2 and T2 and T3 were somewhat similar with both pairs of trials demonstrating ~ 25/75% chance of substantial change and negligible difference, respectively. However, the standardised change in mean (i.e. effect size) was lowest for T2 vs. T3, further demonstrating T2 and T3 had the strongest relationship (Table 4.2, Figure 4.2).

Table 4.2 Summary of reliability data between trials for T_{LIM}

	T1 vs. T2	**T1 vs. T3**	**T2 vs. T3**
Mean bias (s) ± SE	24 ± 31	40 ± 41	16 ± 20
Limits of Agreement	-63 – 111	-74 – 154	-40 – 71
95% CI	-37 – 86	-41 – 121	-24 – 55
Within-subject CV	10%	14%	7%
Effect Size	0.5	0.6	0.2
r	0.66	0.73	0.97
r^2	0.43	0.53	0.95

Table 4.3 Magnitude based inference data for T_{LIM}

Trial Comparison (A, B)	SWC #	A > B	Negligible	A < B
T1 vs.T3	2.6%	0.0%	62.0%	37.9%
T1 vs. T2	2.6%	0.1%	77.6%	22.4%
T2 vs. T3	2.6%	0.1%	76.6%	23.3%

Note: # SWC calculated as 0.2 * between-subject SD of baseline W_{PEAK}

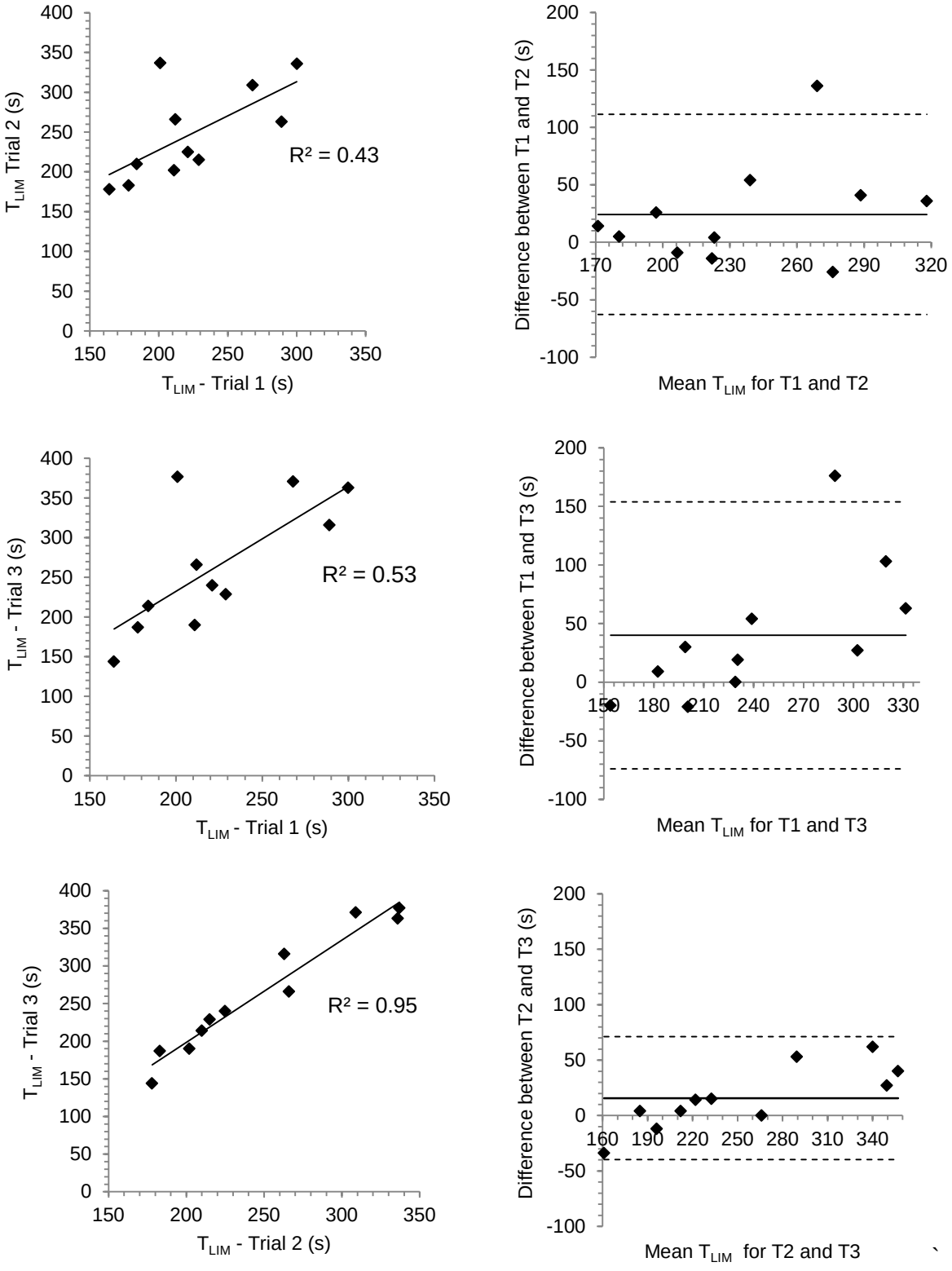

Figure 4.2 Relationship between exercise capacity in each trial (left panel) and Bland-Altman plots (right panel). Top to bottom; T1 vs. T2, T1 vs. T3 and T2 vs. T3). Dotted lines represent limits of agreement (+/- 1.96 SDs; 95% confidence) and solid line is mean bias between trials.

4.5 Discussion

The aim of this study was to determine the familiarisation to and test re-test reliability of constant load cycling at 110% W_{PEAK} in non-specifically cycle trained individuals. These results support our hypothesis that two familiarisation trials are required to minimise systematic bias. Therefore, for the human studies within this thesis (study 2, chapter 5; study 4, chapter7) two familiarisation trials will be undertaken prior to experimental data collection. Although magnitude based inferences suggested there was a 25% chance of substantial change between T2 and T3, correlation coefficients (r), coefficient of determination (r^2), limits of agreement (LoA) and within-subjects CV (CV_{WS}) improved across trials demonstrating T2 and T3 to have the strongest relationship. This is supported by the reduction in effect sizes across trials with effect sizes for T1 vs. T3 and T1 vs. T2 greater than the smallest worthwhile change (Figure 4.2; Table 4.2; Hopkins 2004). These results are supported by previous research which demonstrated that familiarisation to predominantly anaerobic maximal intensity cycling requires two trials (Capriotti, Sherman, and Lamb 1999, Glaister et al. 2003, Mendez-Villaneuva, Bishop, and Hamer 2007). Similar to the present study, participants in these studies were unfamiliar with the exercise protocol used and not specifically cycle trained. Furthermore, the CV_{WS} for the present study (7%) was much lower than that reported in a meta-analysis on the reliability of T_{LIM} (16%; Hopkins, Schabort, and Hawley 2001) as well as that reported elsewhere in studies using T_{LIM} (Hinckson and Hopkins 2005, Amann, Hopkins, and Marcora 2008). In contrast, CV_{WS} in the present study was similar to that reported in highly trained cyclists (Laursen, Shing, and Jenkins 2003).

At the time of starting this study there was no reported research which sufficiently assessed the reliability of T_{LIM} at 110% W_{PEAK}. However, in the interim period research was completed addressing the reliability of constant load cycling at 110% W_{PEAK}. In accordance with the results of the present study Saunders et al. (2012) demonstrated that 2 familiarisation trials are required to afford high reliability before experimental trials. Despite a

similar overall result, mean T_{LIM} for T2 and T3 of the present study (256 ± 70 s) is almost double that reported (135 ± 20 s) by Saunders et al. (2012). Moreover, T_{LIM} for 120% W_{PEAK} in the CON trial of study 2 (170 ± 30 s; chapter 5) was 26% higher than the 135 ± 20 s (110% W_{PEAK}) reported by Saunders et al. (2012). Moreover, in the present study 110% W_{PEAK} was 247 ± 32 W which is considerably lower than the 337 ± 54 W reported by Saunders et al. (2012). As similar participant cohorts were used these differences are most likely due to the different cycle ergometers and incremental test protocols used. Despite such large differences in T_{LIM} between studies, physiological data was very similar. Blood lactate at the end of exercise (12.0 ± 2.1 vs. 12.1 ± 2.1 mmol.l^{-1}), 5 mins post-exercise (11.6 ± 2.5 vs. 11.8 ± 1.9 mmol.l^{-1}) and pH 5 mins post-exercise (7.24 ± 0.05 vs. 7.24 ± 0.05) was almost identical between the present study and Saunders et al. (2012), respectively. Therefore, despite adopting slightly different methods to calculate 110% W_{PEAK} the results of the present study and that of Saunders et al. (2012) were largely similar. Both studies agree that 2 familiarisation trials are required before experimental data collection when evaluating nutritional interventions that modulate acid-base balance (i.e. pH, [HCO_3^-], BE) during exercise.

In the present study blood lactate (BLa) at the end of exercise and 5 mins post-exercise was similar to other studies incorporating supramaximal cycling tests to exhaustion (125% $\dot{V}O_{2MAX}$, Katz et al. 1984, Costill et al. 1984) and the mean reduction in pH of 0.17 units from pre-exercise to 5 mins post-exercise is similar to that reported in other studies using high-intensity cycling (Kozak-Collins, Burke, and Schoene 1994, Saunders et al. 2012). At the end of exercise HR (184 ± 10 bpm^{-1}) was similar to that found in other research using both 105% (187 ± 11 bpm^{-1}) and 115% (186 ± 9 bpm^{-1}) supramaximal cycling (Astorino, White, and Dalleck 2009) and also within the 10 bpm^{-1} of age predicted maximum (191 ± 3 bpm^{-1}; Tanaka, Monahan and Seals 2001) as outlined by Bird and Davison (1997). Moreover, ratings of perceived exertion (RPE) at the end of exercise were the same as those found in supramaximal (120% $\dot{V}O_{2MAX}$) running to exhaustion (Price and Simons

2010). In contrast, despite mean T_{LIM} being almost identical to similar research (Astorino, White, and Dalleck 2009) RER at the end of exercise was lower in the present study (1.10 ± 0.13 vs. 1.35 ± 0.17). However, Astorino, White, and Dalleck (2009) did not report the nutritional approach undertaken by their sedentary participants and exercise capacity had a considerably higher variance, all of which could explain this difference. Therefore, as physiological data (1) meets the requirements set out by Bird and Davison (1997), (2) is similar to other research using a similar exercise intensity and (3) is not significantly different across trials, we can be confident that 2 familiarisation trials are needed to ensure reliable data is collected during subsequent experimental trials.

Weber and Schneider (2001) evaluated the reliability of maximal accumulated oxygen deficit (MAOD) during cycling at 110% and 120% of $\dot{V}O_{2PEAK}$. They observed a similar relationship ($r = 0.95$; $r^2 = 0.90$) to that of the present study when comparing repeated exercise capacity at 110% $\dot{V}O_{2PEAK}$. The authors suggested that this relationship was observed with only one familiarisation trial. However, participants actually completed fourteen exercise sessions before the main experimental trials, the final session being a familiarisation test at either 110% or 120% $\dot{V}O_{2PEAK}$. Furthermore, due to the randomisation of experimental sessions between 110% or 120% $\dot{V}O_{2PEAK,}$ it is likely that participants completing their 110% trials will have done so after completing at least one trial at 120% $\dot{V}O_{2PEAK}$. The increased number of exercise sessions is probably why this study reported a smaller difference in T_{LIM} between trials (1.3%) and lower CV (4%) than the present study (6% and 7% respectively). However, it should be acknowledged that the CV calculated from Weber and Schneider (2001) is between-participants/trials as the within-subjects data was not available. With such a small change in T_{LIM} at 110% $\dot{V}O_{2PEAK}$ (1.3%) it is likely the CV_{WS} would be < 4%.

Two studies examining the reproducibility of T_{LIM} at 100% $\dot{V}O_{2PEAK}$ in trained cyclists established that T_{LIM} was significantly longer in the second trial of two. Laursen, Shing, and

Jenkins (2003) observed a difference of 8 s / 3% (CV_{WS} = 6%) and Costa et al. (2011) reported a difference of 15 s / 7% (CV_{WS} not given). Similarly, Martin, Diedrich, and Coyle (2000) evaluated the time course of learning within maximal cycling over consecutive days. They reported that active but non cycle trained males needed at least two days to demonstrate reliable data for maximal cycling, whereas reliable data was observed after only one day for trained cyclists. Such research suggests that reliable data for trained cyclists is achieved with just one familiarisation trial whereas at least two familiarisation trials are required with unfamiliar/untrained participants. Unfortunately neither study of trained athletes conducted a third trial and therefore it is not possible to ascertain whether further increases in T_{LIM} may occur and thus if more familiarisation trials are required.

Previous research has evaluated cycling at both 105% and 115% $\dot{V}O_{2MAX}$ as methods to confirm attainment of $\dot{V}O_{2MAX}$ in sedentary individuals (Astorino, White, and Dalleck 2009). Although significant correlations for $\dot{V}O_2$ across trials at both 105% and 115% $\dot{V}O_{2MAX}$ were observed, exercise time was not related (r^2 = 0.19) across trials at 115% $\dot{V}O_{2PEAK}$. Similar to the results of the present study this further demonstrates that in non-specifically trained individuals at least 3 trials are required to suitably assess test re-test reliability (Hopkins 2000). Indeed, one limitation of the present study is that a further plateau in T_{LIM} might have occurred after a fourth (or more) trial(s). Moreover, conducting a fourth trial might also result in a reduction in the slight heteroscedasticity between T2 and T3 observed in participants who cycled for the longest durations (Figure 4.2). However, it is well acknowledged that motivation to maintain power output in the face of fatigue is likely to be more variable in longer tests (Hopkins, Schabort, and Hawley 2001). In contrast, O'Hara et al. (2012) reported that trained cyclists, who generally produce more reliable repeated exercise performance than non-trained cyclists (Hopkins, Schabort, and Hawley 2001) had a greater level of variance (demonstrated by limits of agreement data and CV of 32%) over 4 familiarisation trials when evaluating the reliability of T_{LIM} at the end of an endurance cycling test. In contrast, our data demonstrates high reliability between T2 and T3 (r = 0.97, r^2 =

0.95, CV_{WS} = 7%) with only this data falling within the *a priori* limits of agreement (Figure 4.2).

Learning effects on successive trials can be a considerable source of test-retest measurement error (Atkinson and Nevill 1998). Our results demonstrated that in three successive supramaximal cycling trials to volitional exhaustion, a significant improvement in exercise capacity occurred between the first and third trials (18%; 40 s; ES = 0.6). This improvement in capacity was achieved without significant changes in any physiological measurements. Therefore, such improvement may be due, at least in part, to psychological factors outside of experimental manipulation such as conscious or subconscious changes in effort due to recognition of the "final trial" (Hickey et al. 1992, Martin, Diedrich, and Coyle 2000). An improvement in r^2 values across trials culminated in a strong and significant relationship between T2 and T3 and only this relationship demonstrated all data to be within 95% limits of agreement combined with the lowest bias between trials. Finally, the 6% (16 s) difference between T2 and T3 is similar to the differences of 7% (15 s) (Costa et al. 2011) and 3% (8 s) (Laursen, Shing, and Jenkins 2003) in studies comparing reliability at 100% $\dot{V}O_{2PEAK}$ in competitive cyclists. Therefore, by undertaking at least two familiarisation trials the test re-test measurement error in non cycle trained individuals is significantly reduced to ~ 6% / 16 s and therefore high reliability afforded.

To the best of our knowledge there are no guidelines as to what constitutes a valid supramaximal exercise test. Indeed, Hopkins, Schabort, and Hawley (2001) note that no research on the validity of anaerobic performance measures has included reliability of the criterion measure(s), suggesting that tests of high reliability be used as only these tests can have high validity. Our results demonstrate that constant load supramaximal cycling at 110% W_{PEAK} achieves similar maximal physiological values to an incremental exercise test to $\dot{V}O_{2PEAK}$ (Bird and Davison 1997). Furthermore, although there was a significant difference between local fatigue (RPE_L) and cardiovascular fatigue (RPE_O) at volitional exhaustion, the

closeness of the values (20 ± 0 and 19 ± 1 respectively) demonstrate that T_{LIM} in this study was unlikely to be limited by the early onset of localised muscular fatigue which can occur when undertaking unfamiliar exercise (Smith, Price, and Doherty 2001). Moreover, T_{LIM} in the present study is similar to previous studies of male and female non trained participants cycling at 110% $\dot{V}O_{2PEAK}$ (225 s) (Weber and Schneider 2001) and 105% $\dot{V}O_{2PEAK}$ (224 s and 250 s) (Astorino, White, and Dalleck 2009). In contrast, Gastin et al. (1995) reported shorter exercise durations of 186 s and 208 s for 110% W_{PEAK} cycling undertaken at either 90 rev.min^{-1} or 70 rev.min^{-1} in groups of moderately trained or mixed trained and untrained males respectively. The difference in training status and chosen cadence may have contributed to the observed difference(s) in T_{LIM} between Gastin et al. (1995) and the present study.

In summary, the ability to detect changes outside of day-to-day variation in exercise capacity (i.e. sensitivity) is of particular importance in research where an intervention such as nutritional supplementation is evaluated (Hill et al. 2007, Sewell and McGregor 2008, Sale et al, 2011, Saunders et al. 2011, O'Hara et al. 2012, Saunders et al. 2012). Due to the pervasiveness of nutritional supplements within the field of sports science and the clear link to the field of strength and conditioning and physical rehabilitation it is important that researchers and sports practitioners can evaluate exercise capacity data regarding such interventions accurately. Moreover, with a considerable number of nutritional ergogenic aids purchased and consumed by the non-elite athlete it is important to ensure that this population is accurately reflected within the sports science literature. In conclusion, T_{LIM} cycling at 110% W_{PEAK} is a valid and reliable exercise protocol when assessing high intensity exercise capacity with a significant aerobic component. We recommend that at least two familiarisation trials are completed prior to experimental data collection for participants unfamiliar with such exercise. It is our contention that completing two familiarisation tests represents a good balance between scientific control, logistical reality and participant adherence whilst concomitantly minimising the possibility of training effects (Hill et al. 2007,

Sale et al. 2011, Saunders et al. 2012). For the subsequent human studies within this thesis, (study 2 and study 4; chapters 5 and 7, respectively), two familiarisation trials will be undertaken prior to experimental data collection.

Chapter 5 – Evaluating the effects of sodium bicarbonate ($NaHCO_3$) on high intensity cycling capacity

5.1 Abstract

Ten healthy, non-cycling trained males (age: 21.2 ± 2.2 years, body mass: 75.9 ± 13.4 kg, height: 178 ± 6 cm, $\dot{V}O_{2PEAK}$: 46 ± 10 $ml.kg^{-1}.min^{-1}$) performed a graded incremental exercise test, two familiarisation trials and six experimental trials. Experimental trials consisted of cycling to volitional exhaustion at 100%, 110% and 120% W_{PEAK}, 60 mins after ingesting either 0.3 $g.kg^{-1}$ body mass sodium bicarbonate ($NaHCO_3$) or 0.1 $g.kg^{-1}$ body mass sodium chloride (NaCl; PLA). At the group level $NaHCO_3$ ingestion increased capacity (T_{LIM}) by 17% at 100% W_{PEAK} (327 vs. 383 s; P = 0.02) although not at 110% W_{PEAK} (249 vs. 254 s; P = 0.66) or 120% W_{PEAK} (170 vs. 175 s; P = 0.60; PLA and $NaHCO_3$ respectively). However, there was marked inter and intra individual variance at 110% and 120% W_{PEAK}. Heart rate (P = 0.02), blood lactate (P = 0.001), pH (P < 0.001), [HCO_3^-], (P < 0.001), and base excess (P < 0.001) were greater in all $NaHCO_3$ trials. $NaHCO_3$ attenuated localised ratings of perceived exertion (RPE_L) to a greater extent than PLA only at 100% W_{PEAK} (P < 0.02). Ratings of abdominal discomfort and gut fullness were mild but higher for $NaHCO_3$. $NaHCO_3$ ingestion significantly improves continuous constant load cycling at 100% W_{PEAK} due to, in part, attenuation of RPE_L.

5.2 Introduction

The efficacy of sodium bicarbonate ($NaHCO_3$) as an ergogenic aid remains equivocal (Requena et al. 2005, Price and Simons 2010, Saunders et al. 2011). Maughan (1999) suggests the lack of agreement between studies is due to variations in the dosage administered, degree of metabolic alkalosis induced and the intensity, duration and nature of the exercise undertaken. Although the first (0.3 $g.kg^{-1}$ body mass; McNaughton 1992) and second (~ 60/90 mins pre-exercise; Price and Singh 2008, Renfree 2007) aspects have

been addressed, the effects of $NaHCO_3$ on exercise capacity over a range of intensities within the same population have yet to be confirmed (Price and Simons 2010).

Two factors that increase the likelihood of an ergogenic benefit being observed with $NaHCO_3$ ingestion are using a high-intensity exercise protocol of between 1 to 7 minutes (Linderman and Fahey 1991, Linderman and Gosselink 1994, Matson and Tran 1993) and constant load exercise to exhaustion (T_{LIM}; Matson and Tran 1993). However, despite T_{LIM} representing the most reliable type of performance test (Hopkins, Schabort, and Hawley 2001) many studies evaluating the efficacy of $NaHCO_3$ on cycling performance have used either Wingate, repeated sprints or fixed duration 'all-out' protocols which have provided inconsistent results. For example, no ergogenic benefit for $NaHCO_3$ was reported using a fixed cadence (100 $rev.min^{-1}$) 30 s effort (McCartney, Heigenhauser, and Jones 1983) or repeated Wingate protocols (Parry-Billings and MacLaren 1986). In contrast, $NaHCO_3$ has been shown to facilitate improvements in both work done and power output during 5 x 6 s all out sprints separated by 30 s recovery (Bishop et al. 2004) and single Wingate tests (Inbar et al. 1983), although the latter study did not include a familiarisation process which may have affected results. Moreover, $NaHCO_3$ had no effect on critical power, total work done or peak power during a 3 mins all out cycling protocol (~ 100% $\dot{V}O_{2PEAK}$) based on the critical power model (Vanhatalo et al. 2010).

Results using constant power protocols appear more consistent. Exercise capacity (T_{LIM}) at 100% $\dot{V}O_{2MAX}$ was 14% greater with $NaHCO_3$ (MacLaren and Morgan 1985) and more recently, Saunders et al. (2011) found that when participants who suffered GI distress were removed from the analysis, $NaHCO_3$ was shown to enhance total work done by 5% at 110% W_{PEAK}. Similarly, two other studies have demonstrated that T_{LIM} at 125% $\dot{V}O_{2MAX}$ cycling was 42% (Costill et al. 1984) and 45% (McKenzie et al. 1986) longer after $NaHCO_3$ although T_{LIM} was measured on the fifth and sixth bout following an intermittent protocol of repeated 1 min bouts (1 min rest between bouts).

At present, no study has compared the efficacy of $NaHCO_3$ at different exercise intensities within the same population. Such research is important as this might help elucidate the mechanisms for how $NaHCO_3$ affects performance (Price and Simons 2010) and why certain participants appear to respond to $NaHCO_3$ ingestion and others do not (Price and Simons 2010, Saunders et al. 2011). Therefore, the purpose of this study was to compare T_{LIM} cycling capacity at 100%, 110% and 120% W_{PEAK} in the same participants. Exercise at these intensities should sufficiently stress the glycolytic energy system but result in appreciably different capacity times (T_{LIM}) within the 1 to 7 minute window within which $NaHCO_3$ is regarded to be most effective (Linderman and Fahey 1991, Matson and Tran 1993, Linderman and Gosselink 1994). It was hypothesised that $NaHCO_3$ would improve T_{LIM} at all intensities.

5.3 Methods

5.3.1 Participants

Ten healthy and active males volunteered to take part in this study (age 21.2 ± 2.2 years, body mass 75.9 ± 13.4 kg, height 178 ± 6 cm, $\dot{V}O_{2PEAK}$ 46 ± 10 $ml.kg^{-1}.min^{-1}$) which had received University Ethics Committee approval. All participants were recreationally active undertaking 2 to 3 exercise bouts per week (e.g. football, rugby and/or running). None were specifically cycling trained.

5.3.2 Study design

Participants visited the laboratory on nine separate occasions. On the first occasion participants completed an incremental test on a cycle ergometer to determine $\dot{V}O_{2PEAK}$ and peak mean minute power (W_{PEAK}; section 3.6.1). Based on the results from study 1 (chapter 4), on the second and third visits participants undertook two T_{LIM} familiarisation trials at 110% W_{PEAK}. On the subsequent six visits participants cycled to volitional exhaustion at a constant

load equivalent to 100%, 110% or 120% W_{PEAK} at 70 rev.min^{-1}, 60 minutes after consuming either 0.3 g.kg^{-1} body mass $NaHCO_3$ or 0.1 g.kg^{-1} body mass NaCl (PLA) as described in section 3.6.2. Section 3.7 describes the treatment administration in more detail and participant screening and pre-experimental procedures are outlined in sections 3.2 and 3.3. At the end of the study participants completed an achievement goal questionnaire (AGQ; Conroy, Elliot, and Hofer 2003). The AGQ describes different goal orientated approaches that participants might use to achieve competence in sporting activities. They responded on a scale of 1 = “not at all like me” to 7 = “completely like me” (Conroy, Elliot, and Hofer 2003). The results were analysed to determine whether any difference in T_{LIM} performance could be attributed to differences in participant’s self reported achievement goal strategies. A full list of the questions can be found in Table 5.2 (section *5.4.5.iii*).

5.3.3 Protocol overview

After five minutes seated resting heart rate (HR, section 3.3.4), perceived readiness to exercise (PRE; Nurmekivi et al. 2001, section 10.3), abdominal discomfort (AD) and gut fullness (GF; Price, Moss, and Rance 2003, section 3.4.2) were recorded. Blood samples were then taken for blood lactate concentration ([BLa]), pH, base excess (BE) and bicarbonate ion concentration ($[HCO_3^-]$). Blood was collected and analysed as outlined in sections 3.5.1 and 3.5.2. After baseline measurements were completed the participant consumed the $NaHCO_3$ or PLA drink within the first 5 mins of the 60 mins pre-exercise period (Price and Simons 2010). Participants remained seated throughout and were allowed to consume water *ad libitum* to minimise gastrointestinal (GI) discomfort. The mean volume of water consumed was monitored and estimated at ~ 350 ml. Perceived readiness to exercise (PRE), AD and GF were recorded 30 mins and 60 mins following ingestion and HR was recorded and further blood samples taken 60 mins following ingestion. Forty-five minutes after ingestion, participants started breathing into the breath-by-breath gas collection system as previously indicated (section 3.3.3). Baseline data was averaged over

the last sixty seconds of the pre-exercise period and for the last ten seconds of exercise. Expired gas was analysed for calculation of $\dot{V}O_2$ and measurement of $\dot{V}_E$.

After baseline data had been collected the participant started a warm up consisting of cycling at 70 rev.min^{-1} for 4 mins at 50% of the subsequent trial intensity (i.e. 100, 110 or 120% W_{PEAK}), 1 min at 75% of the subsequent trial intensity and then 2 mins at 70 W. This warm-up protocol was chosen to prepare participants for the relevant trial by differentiating the warm up intensity based on subsequent exercise intensity. The typical difference in power output between warm-ups was ~ 25 W for each participant. The participant completed the T_{LIM} test at 100%, 110% or 120% W_{PEAK} as described in section 3.6.2. Ratings of perceived exertion (RPE 6-20, Borg 1982) for local RPE (RPE_L), representing the exercising muscles, and overall RPE (RPE_O), reflective of cardiovascular strain were recorded as described in section 3.4.1. Abdominal discomfort, GF and HR were recorded and blood samples taken for BLa, pH, BE and [HCO_3^-] immediately post-exercise and final blood samples taken 5 mins post-exercise. Upon completion of the test, the participant was encouraged to cycle for 5 mins at 70 W (unloaded cradle) to warm down and avoid syncope.

5.3.4 Statistical analysis

Statistical analysis was completed using PASW (SPSS; v17, Chicago, USA). Statistical significance, normality and homogeneity of variance/sphericity of data was assessed / adjusted as outlined in section 3.8. Exercise capacity (T_{LIM}; s) for 110% and 120% W_{PEAK} was analysed by paired t-tests whereas T_{LIM} for 100% W_{PEAK} was analysed using a Wilcoxon test due to non-normality of data. Exercise capacity data was also analysed for the whole group (n=30; Wilcoxon test) and for specific time groups (T_{LIM} < 5 mins, n=19, paired t-test; >= 5 mins, n=11, Wilcoxon test). All cardiorespiratory, perceptual and blood variables were analysed by 3-way (time * treatment * intensity) repeated measures ANOVA. Where significance was achieved for main effects pairwise comparisons

(least significant difference; LSD) were undertaken. LSD comparisons were chosen as they are the most powerful when analysing 3 levels/groups (Maxwell and Delaney 2004, Cardinal and Aitken 2006, Howell 2007). For interactions, Tukeys' post hoc analysis was undertaken by calculating the difference required between means for significance at the level of $P < 0.05$ (Vincent 1999). The time points considered for HR and blood variables were pre-ingestion (-60), pre-exercise (0), immediately post-exercise and five minutes post-exercise. Respiratory data ($\dot{V}O_2$ and $\dot{V}_E$) was considered at rest and post-exercise.

Values for RPE_L and RPE_O were analysed at 1 min and 2 mins during exercise and at volitional exhaustion. Abdominal discomfort and GF were analysed pre-ingestion, 30 mins post-ingestion, pre-exercise and post-exercise. Finally, PRE was analysed pre-ingestion, 30 mins post-ingestion and pre-exercise. Figures quoted are mean values ± standard deviation unless otherwise stated. Correlation coefficients (Spearman's ρ and Pearson's r for non-parametric and parametric data, respectively) and effect sizes (ES) reported where appropriate. For ANOVA ES are reported as partial η^2 and for between trial comparisons ES was calculated as the change in means divided by the pooled SD of the compared trials. Confidence intervals (95%CI) which define the range for which the true value of the statistic is 95% likely to fall (Watt, Hopkins, and Snow 2002) were calculated as ± 1.96 * SE for individual trials, where SE = SD / √n (Streiner 1996) and for mean difference between trials as ± 1.96 * SE for where SE = SD / √2 (Hopkins 2000). The AGQ data was correlated against affects on T_{LIM}. In addition to the traditional statistical analysis, magnitude based inferences are presented where appropriate (Hopkins et al. 2009). Odds ratios (benefit: harm) are also presented for T_{LIM} data where a value of > 66 (i.e. > 25% chance of benefit and < 0.5% chance of harm; Hopkins 2007) representing the recommended threshold (3.8.1).

5.4 Results

5.4.1 Preliminary tests

$\dot{V}O_2$, $\dot{V}_E$, HR, blood lactate (BLa) and RPE at the end of the peak oxygen uptake test were 3.44 ± 0.85 l.min^{-1}, 117.1 ± 21.7 l.min^{-1}, 186 ± 8 bpm^{-1}, 10.8 ± 1.8 mmol.l^{-1} and 19.9 ± 0.3, respectively. This data supports the criteria for achievement of valid maximal *peak* oxygen uptake tests (Bird and Davison 1997). Mean minute power (W_{PEAK}) was 228 ± 37 W.

5.4.2 Exercise capacity (T_{LIM})

T_{LIM} (± SE) at 100% W_{PEAK} was 17% greater after $NaHCO_3$ ingestion (383 ± 44 vs. 327 ± 29 s; P < 0.02). In contrast, T_{LIM} was not different between $NaHCO_3$ and PLA at 110% W_{PEAK} (254 ± 22 vs. 249 ± 20 s; P = 0.66) or 120% W_{PEAK} (175 ± 14 vs. 170 ± 9 s; P = 0.60; Table 5.1, Figure 5.1). Although there were no group level differences at 110% W_{PEAK} and 120% W_{PEAK}, individual performance was variable (Table 5.2).

Table 5.1 Differences in T_{LIM} between $NaHCO_3$ and PLA trials at 100%, 110% and 120% W_{PEAK}

	100% W_{PEAK}	110% W_{PEAK}	120% W_{PEAK}
Mean difference (s) ± SE	57 ± 44 *	5 ± 24	5 ± 21
95%CI (s)	-30 – 143	-42 – 52	-35 – 46
Effect Size	0.5	0.1	0.1

Note: * Significantly different from PLA (P = 0.02). CI represents confidence interval

Table 5.2 Individual level comparisons of exercise capacity (T_{LIM}) at each exercise intensity

Participant	PLA100	SBC100	PLA110	SBC110	PLA120	SBC120	SBC / PLA (% Change)		
1	195	228	157	177	119	118	**17%**	**13%**	-0.8%
2	303	378	308	369	176	130	**25%**	**20%**	-26%
3	550	757	368	385	203	253	**38%**	5%	**25%**
4	375	337	249	250	188	219	-10%	0.4%	**16%**
5	329	360	257	248	199	184	**9%**	-4%	-8%
6	307	330	228	211	187	181	**7%**	-7%	-3%
7	280	353	245	236	133	154	**26%**	-4%	**16%**
8	313	371	291	225	195	210	**19%**	-23%	**8%**
9	296	358	216	251	160	134	**21%**	**16%**	-16%
10	317	358	172	188	141	169	**13%**	**9%**	**20%**
Mean	327	383	249	254	170	175	17%	2%	3%
SE	29	44	20	22	9	14	4%	4%	5%

Note: * All T_{LIM} is in seconds. % change figures in **bold** represent higher than daily variation (6%).

The increase in T_{LIM} of 17% at 100% W_{PEAK} for $NaHCO_3$ equated to a ~ 40% chance of beneficial change. In contrast chances of beneficial change for $NaHCO_3$ at 110% W_{PEAK} and 120% W_{PEAK} were 1.1% and 1.3%, respectively. The benefit to harm odds-ratios of 4 and 6 respectively, were much lower than the 6702 observed for the difference in T_{LIM} at 100% W_{PEAK} (Table 5.3).

Table 5.3 Probability of beneficial, trivial or harmful outcomes for 100%, 110% and 120%

Intensity	SWC *	Beneficial	Trivial	Harmful	Benefit: Harm Odds Ratio
100% W_{PEAK} #	3.2%	37.5%	62.4%	0.0%	6702
110% W_{PEAK}	3.2%	1.1%	98.6%	0.3%	4
120% W_{PEAK}	3.2%	1.3%	98.4%	0.2%	6

Note: * SWC based on 0.2 x B-S SD of initial incremental test; # A separate t-test was run for 100% W_{PEAK} to ensure compatibility with the magnitude based inference analysis

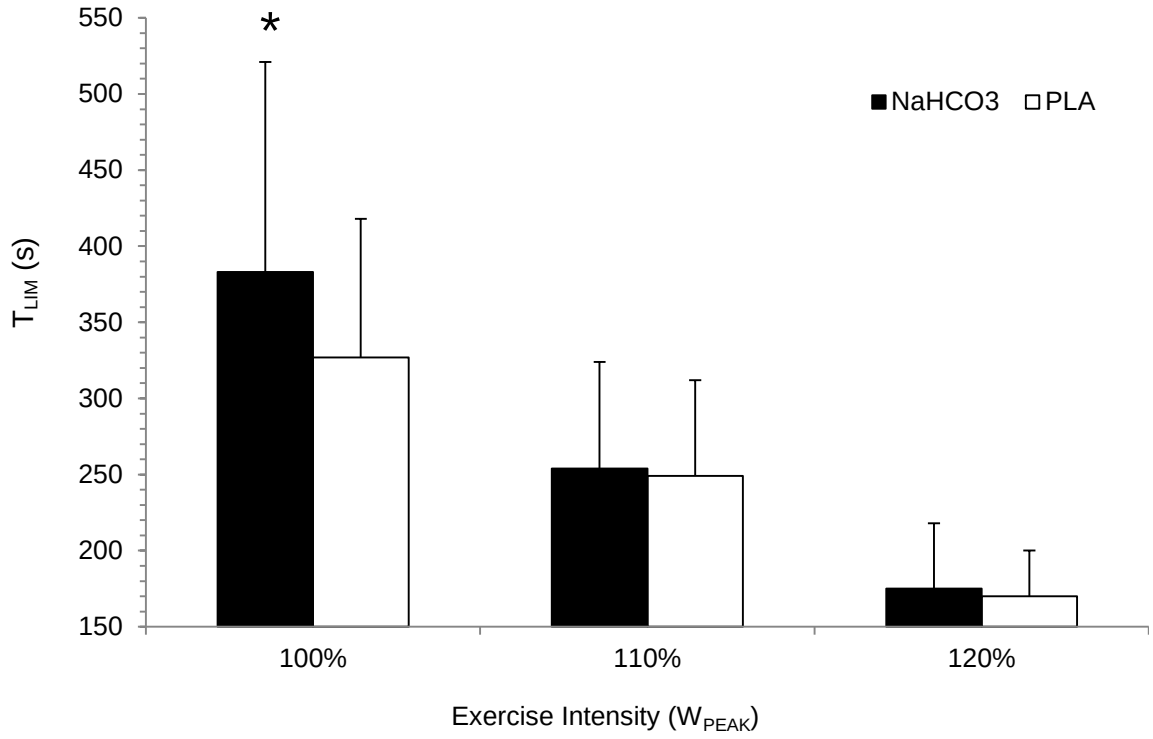

Figure 5.1 Effect of $NaHCO_3$ treatment on T_{LIM} at 100%, 110% and 120% W_{PEAK} (± SD) * P = 0.02)

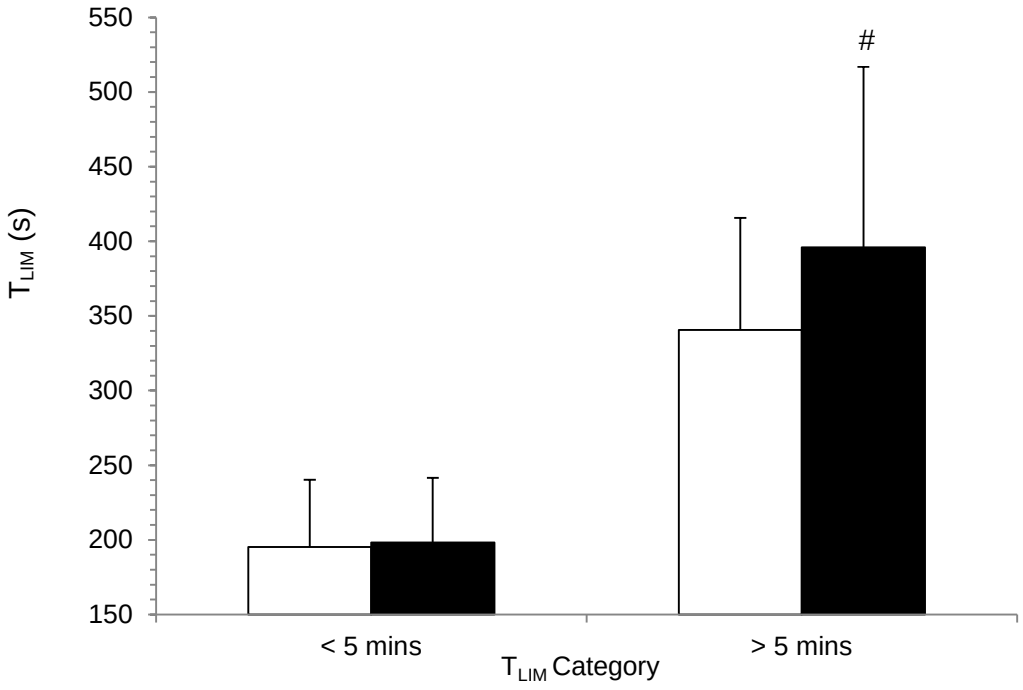

Figure 5.2 Effect of $NaHCO_3$ treatment on T_{LIM} (± SD) split by time category; $^{\#}$ P = 0.01).

Overall exercise capacity was 9% greater for $NaHCO_3$ (P = 0.01; ES = 0.2) with T_{LIM} (± SE) of 271 ± 23 vs. 249 ± 17 s for $NaHCO_3$ and PLA respectively. When grouped for time T_{LIM} was 16% greater (55 s; P = 0.01, ES = 0.6) after $NaHCO_3$ when T_{LIM} was more than 5 mins but no difference between treatments was observed when T_{LIM} was less than 5 mins (3 s; P = 0.67, ES = 0.1; Figure 5.2). No order effect was found between trials (P = 0.09).

Table 5.4 outlines a summary of each 3-way ANOVA. Briefly, a time * treatment * intensity interaction was only observed for blood pH. Time * treatment interactions were observed for [BLa], BE, [HCO_3^-], AD, GF and RPE_L and intensity * time interactions for HR, RPE_L and RPE_O. Only one intensity * treatment interaction was observed (RPE_L).

Table 5.4 Summary of statistical interactions

Interactions				
Measure	Int * Treat	Int * Time	Time * Treat	Int * Time * Treat
HR	x	✓	x	x
BLa	x	x	✓✓	x
pH	x	x	✓✓✓	###
BE	x	x	✓✓✓	x
[HCO_3^-]	x	x	✓✓✓	x
AD	x	x	✓✓✓	x
GF	x	x	+++	x
PRE	x	x	x	x
RPE_L	✓	✓✓✓	✓✓	x
RPE_O	x	✓✓✓	x	x
$\dot{V}O_2$	x	x	x	x
$\dot{V}_E$	x	x	x	x
RER	x	✓	✓	x

Note: ✓ (P < 0.05); ✓✓ (P < 0.01); ✓✓✓ (P < 0.001); ### (P = 0.01); +++ (P = 0.06); x (N/S).

5.4.3 Cardio-respiratory variables

A main effect for treatment ($P = 0.02$; ES = 0.5) and an intensity * time interaction ($P = 0.02$; ES = 0.2) were observed for HR. $NaHCO_3$ ingestion elevated HR when compared to PLA by 5 bpm^{-1} ($P = 0.001$) pre-exercise and at volitional exhaustion (72 ± 11 vs. 67 ± 10 and 185 ± 9 vs. 180 ± 9 bpm^{-1}, respectively). HR at volitional exhaustion was lower at 120% than 110% W_{PEAK} ($P < 0.05$; 180 ± 9 bpm^{-1}, 184 ± 9 bpm^{-1}, respectively) but not at 100% W_{PEAK} (183 ± 9 bpm^{-1}). Main effects for time were observed for both $\dot{V}_E$ ($P < 0.001$; ES = 1.0) and $\dot{V}O_2$ ($P < 0.001$; ES = 1.0).

5.4.4 Blood variables

A treatment * time interaction was observed for BLa ($P = 0.002$; ES = 0.6). At the end of exercise, BLa was greater ($P < 0.01$) for $NaHCO_3$ (14.1 ± 2.3 $mmol.l^{-1}$) than PLA (11.9 ± 2.3 $mmol.l^{-1}$). A significant correlation was observed for the difference in T_{LIM} and difference in BLa at the end of exercise between treatments at 110% ($r = 0.71$; $P = 0.02$) but not at 100% ($\rho = 0.30$; $P = 0.41$) and 120% W_{PEAK} ($r = 0.54$; $P = 0.11$).

There was an intensity * treatment * time interaction for pH ($P = 0.01$; ES = 0.3). There was no difference in pH between any trial pre-ingestion (PLA; 7.42 ± 0.02 vs. 7.41 ± 0.03 vs. 7.42 ± 0.01; $NaHCO_3$; 7.42 ± 0.01 vs. 7.41 ± 0.01vs. 7.41 ± 0.01). Post-ingestion pH was similarly elevated from baseline with $NaHCO_3$ ingestion (7.47 ± 0.01 vs. 7.47 ± 0.03 vs. 7.48 ± 0.02; 100%, 110% and 120% W_{PEAK}, respectively). There was a decrease in pH during the ingestion period for PLA. At the end of the ingestion period pH dropped 0.03 units (7.42 ± 0.02 to 7.39 ± 0.02; $P < 0.001$; ES = 1.3) although remained within normal physiological range (7.38 – 7.42). There were no differences in pH between intensities for $NaHCO_3$ post-exercise (7.30 – 7.31) or 5 mins post-exercise (7.33 – 7.34). However, pH was significantly greater post-exercise for 100% compared to 110% W_{PEAK} ($P < 0.05$; 7.25 ± 0.03

vs. 7.22 ± 0.03) and 5 mins post-exercise (P < 0.01; 7.27 ± 0.04 vs.7.23 ± 0.04) for PLA (Figure 5.3).

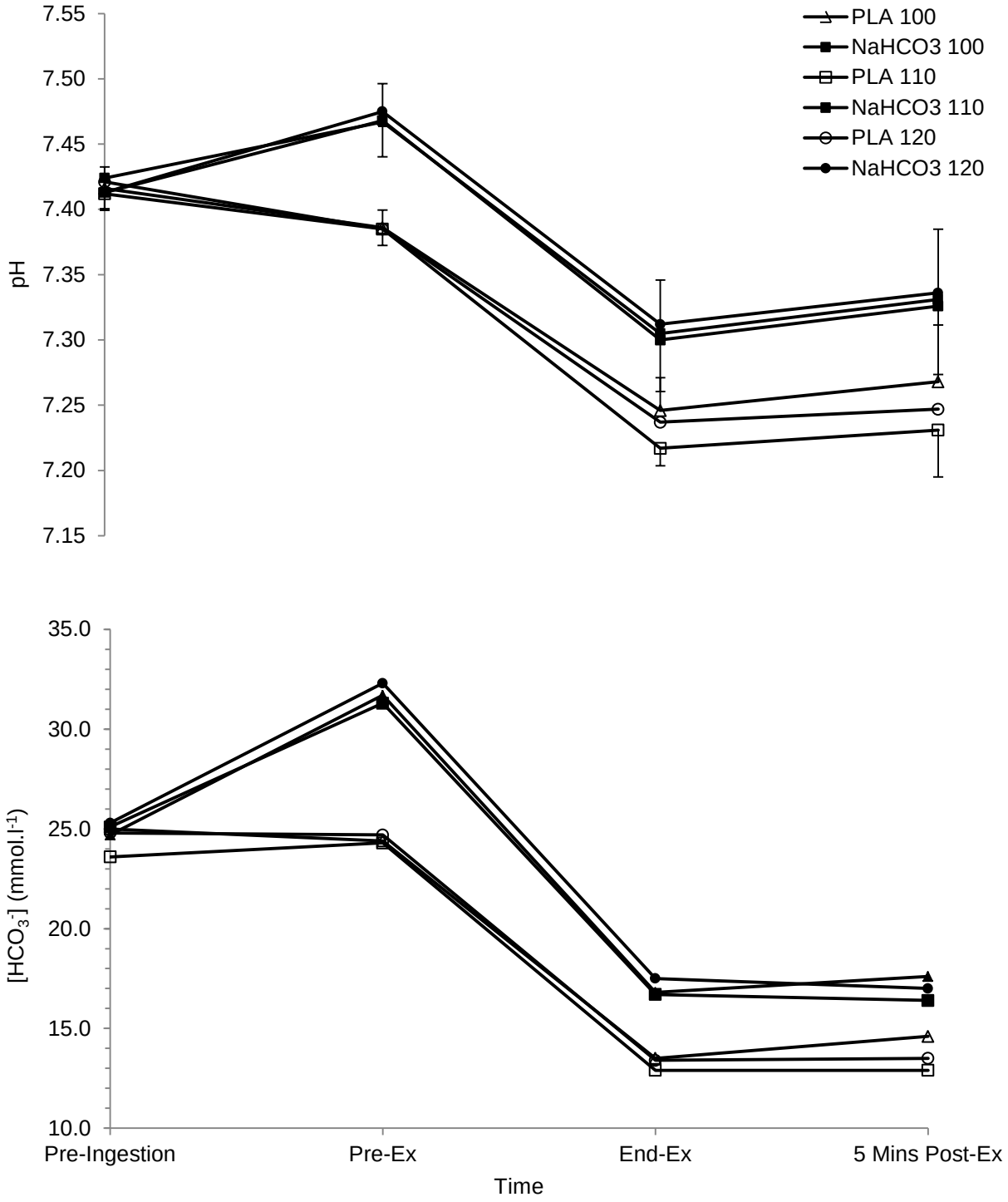

Figure 5.3 pH, and [HCO_3^-] pre ingestion, during absorption, pre-exercise and end-exercise (Error bars represent SD. Some have been omitted for clarity).

Table 5.5 Base excess (BE) and [HCO_3^-] values for $NaHCO_3$ and PLA pre-exercise, at the end of exercise and 5 mins post-exercise.

	BE ($mmol.l^{-1}$)		[HCO_3^-] $mmol.l^{-1}$	
	$NaHCO_3$	PLA	$NaHCO_3$	PLA
Pre-exercise	7.4 ± 2.1	-0.1 ± 1.4	31.8 ± 2.2	24.5 ± 1.4
End-exercise	-8.1 ± 2.5	-13.0 ± 2.2	17.0 ± 2.0	13.3 ± 1.7
5 mins post-exercise	-7.5 ± 3.3	-12.4 ± 3.1	17.0 ± 2.7	13.7 ± 2.3

There were time * treatment interactions for BE ($P < 0.001$; ES = 0.9) and [HCO_3^-] ($P < 0.001$; ES = 0.9). Although similar at rest, BE and [HCO_3^-] were greater pre-exercise, post-exercise and 5 mins post-exercise after $NaHCO_3$ (all $P < 0.01$, Table 5.5, Figure 5.3).

5.4.5 Perceptual variables

5.4.5.i Abdominal discomfort (AD), gut fullness (GF) and perceived readiness to exercise (PRE)

A time * treatment interaction ($P < 0.001$; ES = 0.6) was observed for abdominal discomfort (AD). Overall, AD was 1.5 units higher with $NaHCO_3$ with differences observed at 30 mins post-ingestion (0.7 ± 0.8 vs. 3.0 ± 2.4, $P < 0.01$), pre-exercise (0.6 ± 0.6 vs. 2.7 ± 2.0, $P < 0.01$) and post-exercise (0.9 ± 1.0 vs. 2.3 ± 1.8, $P < 0.01$), respectively. Gut fullness (GF) was not different between treatments ($P = 0.13$) or intensity ($P = 0.43$). However, there was a main effect for time ($P = 0.02$; ES = 0.4) and the time * treatment interaction approached significance ($P = 0.055$; ES = 0.3). The highest level of GF was recorded 30 mins post-ingestion (3.0 ± 2.0 vs. 2.1 ± 2.7; $NaHCO_3$ and PLA respectively) and coincided with peak AD. Abdominal discomfort and GF demonstrated modest correlations at 30 mins post-ingestion ($\rho = 0.30$, $P < 0.02$) and pre-exercise ($\rho = 0.26$; $P = 0.04$). There were no differences observed for PRE.

5.4.5.ii Ratings of perceived exertion (RPE)

There were intensity * treatment (P = 0.02; ES = 0.4), time * treatment (P = 0.007; ES = 0.4) and intensity * time (P < 0.001; ES = 0.7) interactions for localised RPE (RPE_L). Overall, $NaHCO_3$ attenuated RPE_L to a greater extent than PLA at 100% W_{PEAK} after 1 min (P < 0.01; 12.8 ± 2.1 vs.14.3 ± 1.5) and 2 mins (P < 0.01; 14.6 ± 1.8 vs.16.1 ± 1.6) of exercise. Although RPE_L was similar at the end of exercise for each intensity RPE_L was higher (P < 0.01) after 1 min during 120% W_{PEAK} (15.4 ± 1.9) compared to 100% (13.6 ± 2.0) and 110% W_{PEAK} (13.7 ± 1.9). RPE_L also increased (P < 0.05) with exercise intensity after 2 mins (15.4 ± 1.8, 16.3 ± 1.5 and 18.1 ± 1.5 for 100%, 110% and 120% W_{PEAK} respectively; Figure 5.4). Correlations were observed between T_{LIM} and RPE_L after 1 min and 2 mins of exercise for $NaHCO_3$ (r = -0.38; P = 0.04; r = -0.61; P < 0.001, respectively).

There was an intensity * time interaction for overall RPE (RPE_O; P < 0.001; ES = 0.5). Although there was no difference at the end of exercise between exercise intensities (17.6 ± 2.6) RPE_O was higher (P < 0.01) after 1 min and 2 min for 120% (13.2 ± 2.6 and 15.7 ± 2.3) compared to 100% W_{PEAK} (11.7 ± 2.3 and 13.5 ± 2.5) and 110% W_{PEAK} (12.1 ± 2.5 and 14.4 ± 2.5; Figure 5.5). No correlations were observed between T_{LIM} and RPE_O.

5.4.5.iii Achievement goal questionnaire (AGQ)

Table 5.6 highlights the complete results from the achievement goal questionnaire (AGQ), by participant. Table 5.7 highlights the significant correlations that were observed between AGQ responses and the absolute difference in T_{LIM} (s) between treatments and the % difference in T_{LIM} between treatments, at 100%, 110% and 120% W_{PEAK}. Although significant correlations were observed there was no consistent pattern for differences in T_{LIM} between participants at 100% and 110% and 120% W_{PEAK} (Tables 5.2, 5.7).

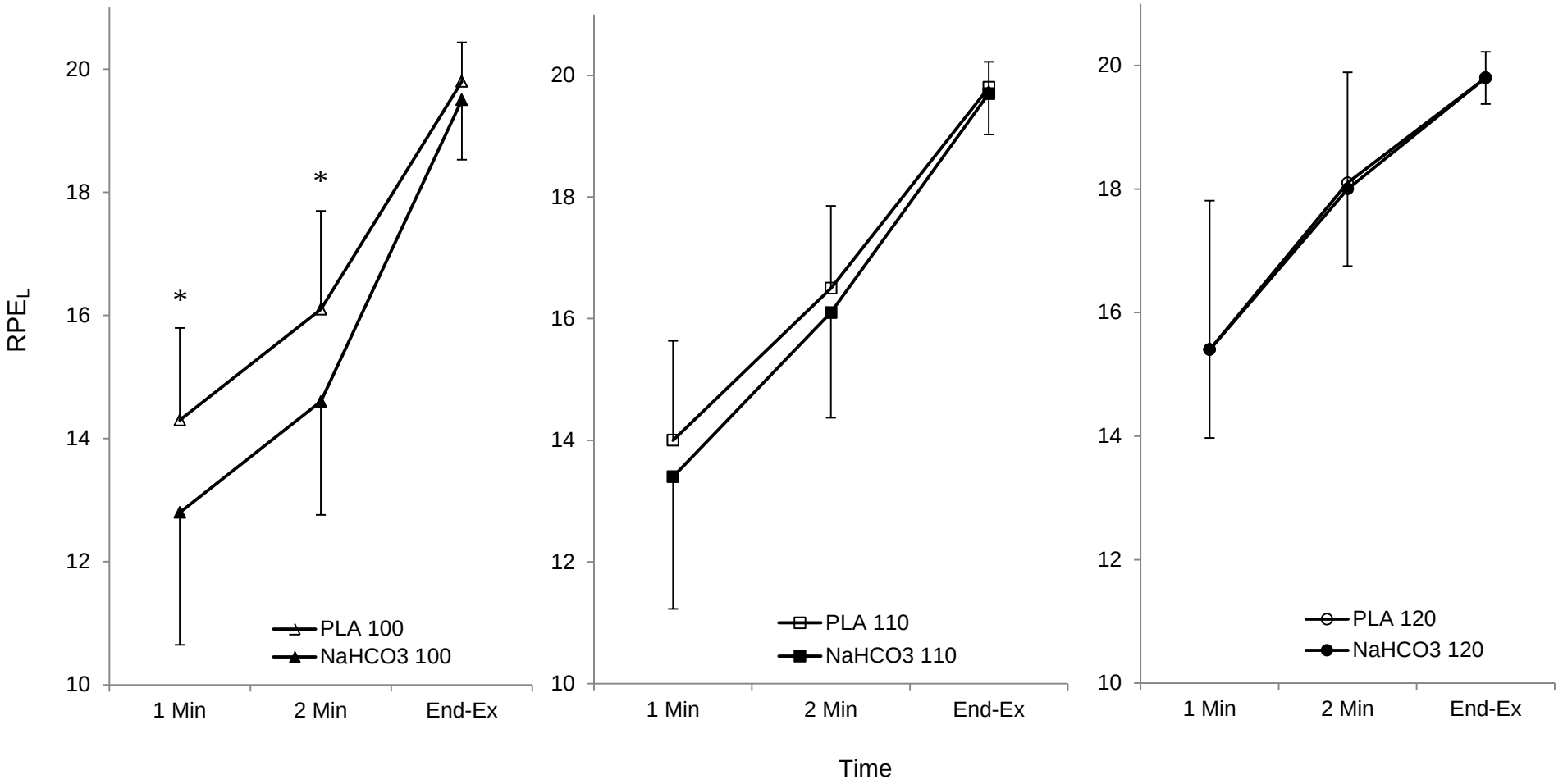

Figure 5.4 Local RPE (RPE_L) between treatments after 1 and 2 mins of exercise and at the end of exercise for 100% (left), 110% (middle) and 120% (right) W_{PEAK} (* $P < 0.01$).

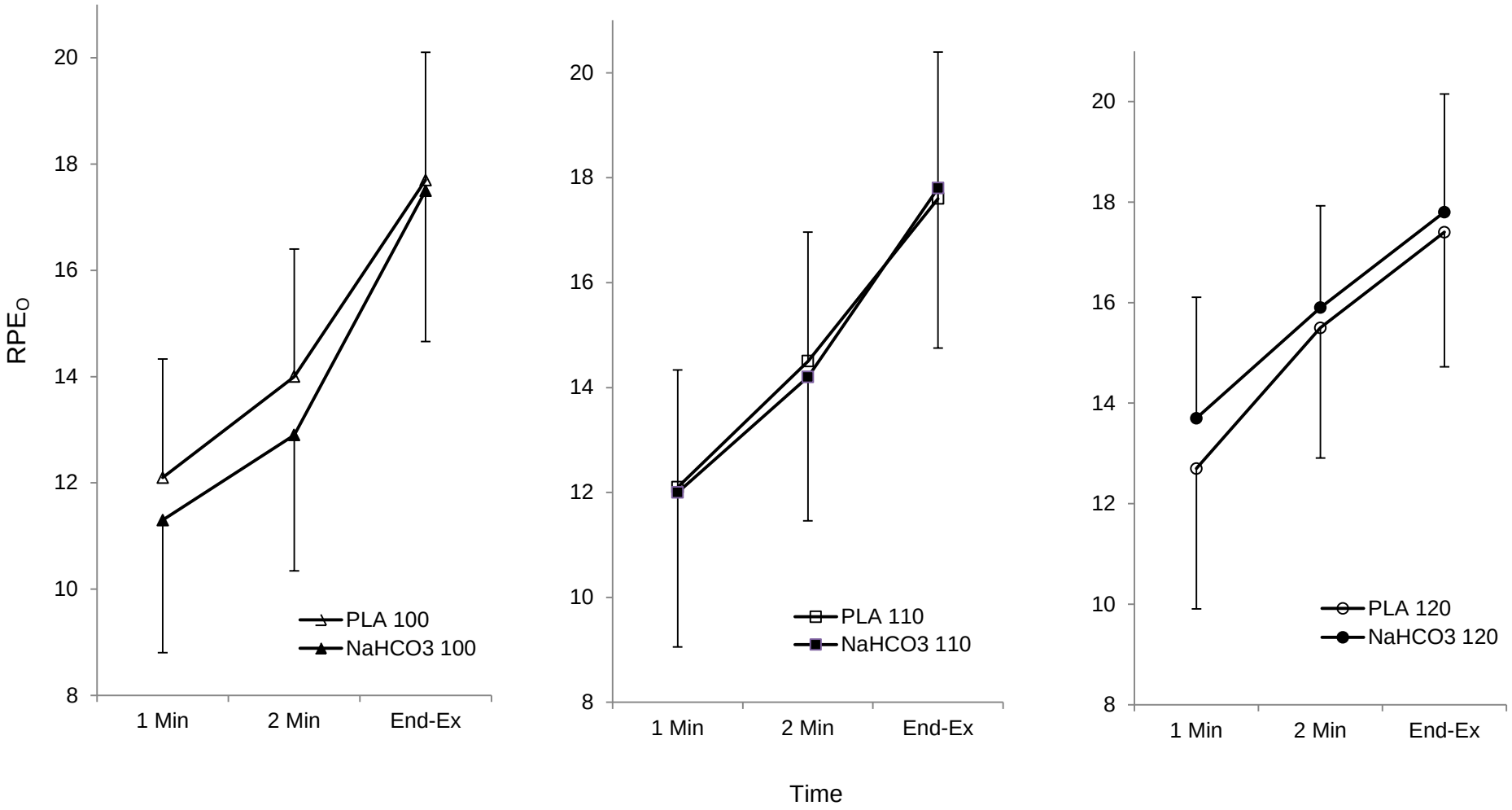

Figure 5.5 Overall RPE (RPE_O) between treatments after 1 and 2 mins of exercise and at the end of exercise for 100% (left), 110% (middle) and 120% (right) W_{PEAK}.

Table 5.6 Achievement goal questionnaire (AGQ) results by participant

Section 1: Mastery Approach	1	2	3	4	5	6	7	8	9	10	Q Mean	SD
(1) It is important to me to perform as well as I possibly can	6	7	7	5	7	7	6	7	7	7	6.6	0.7
(2) I want to perform as well as it is possible for me to perform	7	7	7	5	7	7	6	7	7	7	6.7	0.7
(3) It is important for me to master all aspects of my performance	6	7	6	4	6	4	5	6	7	6	5.7	1.1
Participant Mean	6.3	7.0	6.7	4.7	6.7	6.0	5.7	6.7	7.0	6.7		
SD	0.6	0.0	0.6	0.6	0.6	1.7	0.6	0.6	0.0	0.6		

Section 2: Mastery Avoidance	1	2	3	4	5	6	7	8	9	10	Q Mean	SD
(4) I worry that I may not perform as well as I possibly can	4	3	6	3	4	6	6	5	2	3	4.2	1.5
(5) Sometimes I am afraid that I may not perform as well as I would like	3	5	6	1	3	6	6	6	4	3	4.3	1.8
(6) I'm often concerned that I may not perform as well as I can perform	3	2	6	4	3	7	6	5	1	4	4.1	1.9
Participant Mean	3.3	3.3	6.0	2.7	3.3	6.3	6.0	5.3	2.3	3.3		
SD	0.6	1.5	0.0	1.5	0.6	0.6	0.0	0.6	1.5	0.6		

Section 3: Performance Approach	1	2	3	4	5	6	7	8	9	10	Q Mean	SD
(7) It is important to me to do well compared to others	5	6	4	6	7	6	4	6	6	2	5.2	1.5
(8) It is important for me to perform better than others	5	5	4	5	7	5	3	5	4	2	4.5	1.4
(9) My goal is to do better than most other performers	5	5	5	2	5	5	2	4	7	3	4.3	1.6
Participant Mean	5.0	5.3	4.3	4.3	6.3	5.3	3.0	5.0	5.7	2.3		
SD	0.0	0.6	0.6	2.1	1.2	0.6	1.0	1.0	1.5	0.6		

Section 4: Performance Avoidance	1	2	3	4	5	6	7	8	9	10	Q Mean	SD
(10) I just want to avoid performing worse than others	2	2	1	4	1	4	2	3	1	2	2.2	1.1
(11) My goal is to avoid performing worse than everyone else	1	1	1	1	1	5	5	3	1	2	2.1	1.7
(12) It is important for me to avoid being one of the worst performers in the group	4	2	3	5	1	6	5	5	2	3	3.6	1.6
Participant Mean	2.3	1.7	1.7	3.3	1.0	5.0	4.0	3.7	1.3	2.3		
SD	1.5	0.6	1.2	2.1	0.0	1.0	1.7	1.2	0.6	0.6		

Table 5.7 Significant correlations between AGQ responses and absolute differences in T_{LIM} (top) and % differences in T_{LIM} (bottom) at 100%, 110% and 120% W_{PEAK} (* = Pearson's r, # = Spearman's ρ)

Diff (s)	Q3	Q5	Q6	Q7	Q9	Q11
100%	N/A	N/A	N/A	N/A	N/A	N/A
110%	0.68, P = 0.03 #	N/A	N/A	N/A	N/A	0.71, P = 0.02 #
120%	N/A	N/A	0.64, P < 0.05 *	N/A	N/A	N/A

Diff (%)	Q3	Q5	Q6	Q7	Q9	Q11
100%	N/A	0.71, P = 0.02 *	N/A	N/A	N/A	N/A
110%	0.68, P = 0.03 #	N/A	0.64, P < 0.05 *	N/A	N/A	0.66, P = 0.04 #
120%	N/A	N/A	0.65, P = 0.04 *	0.73, P = 0.02 #	0.66, P = 0.04 *	N/A

5.5 Discussion

This study evaluated the effects of $NaHCO_3$ in the same participants across the range of maximal and supramaximal exercise intensities reported in previous studies. All experimental trials achieved the peak physiological values reported in the preliminary incremental test and thus represent valid (supra)maximal work. In contrast to our original hypothesis, the efficacy of $NaHCO_3$ differed between exercise intensities. At the group level $NaHCO_3$ ingestion facilitated augmented exercise capacity only when exercising at 100% W_{PEAK} with participants cycling for 17% longer (~ 1 min) than PLA. The increase in T_{LIM} of 17% at 100% W_{PEAK} for $NaHCO_3$ equated to a ~ 40% chance of beneficial change.. In contrast chances of beneficial change for $NaHCO_3$ at 110% W_{PEAK} and 120% W_{PEAK} were 1.1% and 1.3%, respectively. The benefit to harm odds-ratios of 4 and 6 respectively, were much lower than the 6702 observed for the difference in T_{LIM} at 100% W_{PEAK} (Table 5.3). Based on the recommended benefit to harm odds-ratio of > 66 $NaHCO_3$ ingestion would be recommended for T_{LIM} at 100% W_{PEAK} but not at 110% or 120% W_{PEAK} (Hopkins 2007,

Hopkins et al. 2009). The 17% increase in T_{LIM} at 100% W_{PEAK} is also considerably greater than the daily variation observed in pilot testing and suggests that $NaHCO_3$ supplementation can be important in improving exercise capacity where T_{LIM} lasts between 5 and 10 minutes. Such a finding is in contrast to the 1 to 7 minute window previously reported (Linderman and Fahey 1991, Matson and Tran 1993, Linderman and Gosselink 1994).

Improvements in exercise capacity at 100% W_{PEAK} were observed in nine of the ten participants. However, capacity at 110% and 120% W_{PEAK} was more variable with 40% and 50% of participants showing improved T_{LIM}. Interestingly, all participants demonstrated improved capacity after $NaHCO_3$ for at least one intensity, 70% demonstrated improvement at two intensities and one participant improved at all three intensities. Interestingly, 30% of participants demonstrated opposing exercise capacity at 110% and 120% W_{PEAK}. Combined exercise capacity data demonstrated an overall ergogenic benefit for $NaHCO_3$ of 9% which is also higher than the daily variation in T_{LIM} observed in pilot testing. It is possible that part of this variation may be explained by the untrained nature of the participants involved, although all participants completed two full familiarisation sessions before experimental trials as described in study 1 (chapter 4). Furthermore, based on the AGQ data, it would seem that variance in T_{LIM} performance at different exercise intensities is not explained by goal orientation. Such variability of performance with $NaHCO_3$ ingestion has been previously reported suggesting that participants were either ‘responders’ or ‘non-responders’ (Price and Simons 2010, Saunders et al. 2011), a method previously used to classify responses to other ergogenic aids (Syrotuik and Bell 2004). However, such research only examined the effects of $NaHCO_3$ on performance at one exercise intensity. The results of the present study are, to the best of our knowledge, the first to challenge this assumption and suggest that such a classification for $NaHCO_3$ ingestion is too simplistic. Such variation in performance after $NaHCO_3$ is in accordance with the results of studies 3 and 4 (chapters 6 and 7, respectively). Further investigation to understand the mechanisms involved is warranted.

Differences in exercise capacity at different exercise intensities might be explained by the rate of change in pH (Lavender and Bird 1989, Price, Moss, and Rance 2003). Indeed, Messonier et al. (2007) observed that greater power output was achieved when the rate of decrease in intramuscular pH was slowest, although this relationship has been questioned (Bishop 2007). Assuming the rate of change in pH increases with exercise intensity this might explain why capacity was greater and RPE_L was attenuated only at 100% W_{PEAK} with $NaHCO_3$ ingestion. Furthermore this might explain why a greater magnitude of ergogenic benefit has been observed for $NaHCO_3$ during exercise of 4 to 8 minutes compared to 1 to 4 minutes (Matson and Tran 1993). Moreover, it's possible that after a certain threshold of pH change, membrane transporters such as monocarboxylate transporter 1 (MCT1), monocarboxylate transporter 4 (MCT4) or the sodium-hydrogen exchanger (NHE) become saturated and therefore less effective. Further research is warranted, particularly on the rate of change in pH, during exercise, with and without $NaHCO_3$.

Messonier et al. (2007) found that participants who had the lowest work capacity during CON trials gained the most benefit from alkalotic buffering, possibly because of lower levels of MCT1 and MCT4. Assuming no significant changes in MCT1 and MCT4 between trials in the current study our results suggest that the roles of such transporters are less clear. In the present study, participants who improved exercise capacity with $NaHCO_3$ at 110% W_{PEAK} had a greater initial W_{PEAK} than participants whose capacity was higher with PLA (244 ± 42 W vs. 183 ± 13 W). In contrast, participants who performed better with $NaHCO_3$ at 120% W_{PEAK} had a lower W_{PEAK} than those who improved with PLA (228 ± 18 W vs. 260 ± 34 W). Moreover, participants who demonstrated no difference in T_{LIM} at 110% had a greater W_{PEAK} than participants who showed no difference at 120% (235 ± 12 W vs.181 ± 35 W). This preliminary analysis suggests that performance in baseline trials is not necessarily a reliable predictor of subsequent exercise capacity with $NaHCO_3$ ingestion.

$NaHCO_3$ ingestion attenuated RPE_L after 1 and 2 mins of exercise during the 100% W_{PEAK} trial. T_{LIM} was also significantly correlated with RPE_L but not RPE_O at the same time points. Attenuation of both RPE_L and RPE_O has been observed at 80% (Robertson et al. 1986) and 90% $\dot{V}O_{2MAX}$ (Swank and Robertson 1989) with $NaHCO_3$ ingestion. Peripheral signals of exertion from active muscles are associated with blood acid-base changes (Robertson et al. 1986), supporting the correlation of higher pH values with lower RPE_L ratings. However, such sensory mechanisms may only contribute to these peripheral signals once a specific metabolic ceiling has been reached, such as the lactate threshold where metabolic acidosis is likely minimal (Robertson et al. 1986). Therefore, once this metabolic ceiling is met, peripheral signals of exertion are likely to increase with exercise intensity. As exercise intensity increases, so will the associated biochemical and physiological changes. Therefore, it is possible that RPE_L was only attenuated at 100% W_{PEAK} because the associated biochemical and physiological changes during exercise at 110% and 120% W_{PEAK} occurred at a rate that prevented $NaHCO_3$ from exhibiting enough of an effect to positively influence T_{LIM}. An explanatory mechanism is yet to be fully elucidated but might be linked to the attenuation of perceived exertion by endogenous opioids (Sgherza et al. 2002), which in itself is likely driven by exercise intensity. Similarly it could be that pre-exercise alkalosis attenuates the stress response (i.e. specific heat shock proteins such as HSP72) during exercise (Peart et al. 2011) and concomitantly facilitates the attenuation of RPE_L. However, it should be acknowledged that no performance benefit was observed in the study evaluating the effects of $NaHCO_3$ on HSP72 although the 4 mins ‘all-out’ protocol used is likely to have been a contributory factor (Peart et al. 2011). The observation that RPE_L was greater than RPE_O at each time point is consistent with previous research demonstrating that RPE_L comprises a greater percentage of the overall perception of effort in cycle exercise in previously untrained males (Hetzler et al. 1991, Hampson et al. 2001). Further research is warranted on the role of $NaHCO_3$ and modulation of RPE_L.

Patterns of pH, BE and [HCO_3^-] were largely similar for both treatments, regardless of intensity (i.e. similar absorption patterns and physiological end-points) and comparable to previous research (Price, Moss, and Rance 2003, Price and Simons 2010, Cameron et al. 2010). One exception was the pattern of pH post-exercise. Absolute pH values post-exercise were more variable for PLA (Figure 5.2).This might be due to individual variation in endogenous concentrations of (non-bicarbonate) intracellular buffers such as carnosine (Begum, Cunliffe, and Leveritt 2005). Although somewhat speculative, $NaHCO_3$ administration may normalise intracellular differences between individuals and provide a more uniform biological starting point to compare performance. This might be achieved by facilitating biochemical transport of H^+ and La^- ions in individuals with lower capacity of intracellular buffers and/or MCT1, MCT4 or NHE and thus explain why post-exercise pH regulation with $NaHCO_3$ was more consistent than in PLA trials.

Despite improvement in exercise capacity only occurring at 100% W_{PEAK}, BLa at the end of exercise, and 5 mins post-exercise, was greater by 2.2 $mmol.l^{-1}$ for $NaHCO_3$ compared to PLA at all intensities. This is consistent with previous research which proposed that performance improvement may not occur unless a difference of > 2 $mmol.l^{-1}$ BLa is observed when using $NaHCO_3$ or sodium citrate administration (Ibanez et al. 1995). Despite showing an ergogenic benefit for $NaHCO_3$ in the present study, the correlation between the difference in T_{LIM} and difference in BLa at the end of exercise between treatments at 100% W_{PEAK} was low and insignificant suggesting that enhanced performance is not necessarily greatly contributed to by augmented metabolic flux.

Previous research suggests that performance after $NaHCO_3$ ingestion can be affected by gastrointestinal (GI) distress (Cameron et al. 2010, Saunders et al. 2011). Saunders et al. (2011) found that $NaHCO_3$ did not enhance performance at 110% W_{PEAK}. However, when participants who suffered GI distress (n=4) were removed from the analysis, $NaHCO_3$ was shown to enhance total work done by 5%. In the present study peak ratings of

AD (3.0 vs. 0.7) and GF (3.0 vs. 2.1) were greater for $NaHCO_3$ than PLA trials although these absolute scores represent only mild AD or GF. Consequently, there is minimal likelihood that either AD or GF impacted on the overall results in this study. In contrast, individual scores for both AD and GF ranged from 0 to 8 at 30 mins post-ingestion and 0 to 6 at 60 mins post-ingestion with $NaHCO_3$. Therefore, it is possible that AD and GF impacted at an individual level and only in specific trials. For example, the one participant who did not improve at 100% W_{PEAK} (-10% with $NaHCO_3$) suffered from GI distress in all trials although GI distress did not prevent improvement in exercise capacity at 120% W_{PEAK} (+16%). This improvement occurred during the last trial and was accompanied by lower AD and GF ratings than reported at 100% and 110% W_{PEAK}. Additionally, one participant who improved only at 100% W_{PEAK} (+9%) did so in the last $NaHCO_3$ trial. Despite recording mid-high AD (6.0) after 30 minutes, in line with the 110% and 120% $NaHCO_3$ trials (8.0 and 6.0), AD dropped substantially pre-exercise (2.0 vs. 6.0). Such results suggest that improved GI tolerance to $NaHCO_3$ over time may have contributed to improvements in exercise capacity in those individuals. Nevertheless one participant who reported mid-high (6.0) AD 30 mins pre-exercise and pre-exercise had the highest increase in T_{LIM} (+38%) at 100% W_{PEAK} with $NaHCO_3$. Thus, GI distress does not always negatively influence performance (Price and Simons 2010).

One minor limitation of the present study is that changes in haemoconcentration were not measured. Theoretically, this could have impacted on the differences observed in blood results in the present study. However, we suggest this is very unlikely based on Kozak-Collins, Burke, and Schoene (1994) and Driller et al. (2012a) who noted that although haematocrit increased at the end of exercise there was no difference between $NaHCO_3$ and NaCl trials. These findings suggest that the intravascular fluid status was the same for both trials and that differences in fluid status did not impact results. Moreover, the exercise protocol used by Kozak-Collins, Burke, and Schoene (1994) was far longer than the present

study and is thus more likely to have demonstrated significant changes in haemoconcentration.

In conclusion, the results of this study suggest that $NaHCO_3$ ingestion improves continuous constant load cycling at 100% W_{PEAK}. Exercise at other intensities (110% and 120% W_{PEAK}) also resulted in improvement in exercise capacity in some participants but this appears more variable. More research is required to elucidate the mechanisms for why improvement in exercise capacity with $NaHCO_3$ ingestion for the same population may differ between exercise intensity. Specifically future research should focus on the role of localised RPE, the rate of pH change during exercise, whether increased tolerance to $NaHCO_3$ influences exercise capacity and whether a change in training status affects the efficacy of $NaHCO_3$ as an ergogenic aid.

Chapter 6 – The effects of elevated levels of sodium bicarbonate ($NaHCO_3$) on the acute power output and time to fatigue of maximally stimulated mouse soleus and extensor digitorum longus muscles

6.1 Abstract

This study examined the effects of elevated buffer capacity (~ 32mM [HCO_3^-]) through administration of sodium bicarbonate ($NaHCO_3$) on maximally stimulated isolated mouse soleus (SOL) and extensor digitorum longus (EDL) muscles undergoing cyclical length changes at 37°C. The elevated buffering capacity was of an equivalent level to that achieved in humans with acute oral supplementation. The acute effects of elevated [HCO_3^-] were evaluated on (1) maximal acute power output (PO) and (2) time to fatigue to 60% of maximum CON PO (T_{LIM60}), the level of decline in muscle PO observed in humans undertaking similar exercise, using the work loop technique. Acute PO was on average 7.0 ± 4.8 % greater for $NaHCO_3$ treated EDL muscles ($P < 0.001$; ES = 2.0) and 3.6 ± 1.8 % greater for $NaHCO_3$ treated SOL muscles ($P < 0.001$; ES = 2.3) compared to CON. Increases in PO were due to greater force production throughout shortening. The acute effects of $NaHCO_3$ on EDL were significantly greater ($P < 0.001$; ES = 0.9) than on SOL. Treatment of EDL ($P = 0.22$; ES = 0.6) and SOL ($P = 0.19$; ES = 0.9) did not alter the pattern of fatigue. Although significant differences were not observed in whole group data, the fatigability of muscle performance was variable suggesting, that there may be inter-individual differences in response to $NaHCO_3$ supplementation. These results present the best indication to date that $NaHCO_3$ has direct peripheral effects on mammalian skeletal muscle resulting in increased acute power output.

6.2 Introduction

In the early part of the 20th century, research suggested that lactic acid accumulation was a predominant cause of skeletal muscle fatigue in intact (Fletcher and Hopkins 1907) and isolated skeletal muscle (Hill and Kupaloc 1929), such fatigue occurring most rapidly under anaerobic conditions and during rapid contractions, respectively. Based on the understanding that lactic acid accumulation during exercise could be, at least in part, neutralised by extracellular buffers, Dennig et al. (1931) evaluated the effects of a known alkalotic buffer on exercise performance. It was reported that orally ingested sodium bicarbonate ($NaHCO_3$) improved running performance.

Contemporary biochemical understanding suggests that during high-intensity exercise there is a concomitant increase of La^- and H^+ ions in both working muscle and blood. This is due to the accumulation and subsequent disassociation of lactic acid (Requena et al. 2005, Thomas et al. 2005). The increase in H^+ (as opposed to accumulation of lactic acid *per se*) and subsequent decrease in pH has been implicated in the cause of muscle fatigue (Requena et al. 2005, Allen, Lamb, and Westerblad 2008). Therefore, augmenting muscle's ability to neutralise excess H^+, through ingestion of alkalotic buffers, may prolong exercise capacity (Begum, Cunliffe, and Leveritt 2005).

The mechanism by which $NaHCO_3$ ingestion is thought to modulate exercise performance is by increasing extracellular buffering capacity, facilitating greater efflux of La^- and H^+ from the working muscle and thus facilitating prolonged muscular contraction and hence exercise performance (Requena et al. 2005). Numerous studies in humans have examined the effect of alkalotic buffers such as $NaHCO_3$ on whole body exercise performance (MacLaren and Morgan 1985, McNaughton 1992a, Lindh et al. 2008). However, results in human studies remain somewhat equivocal due to factors such as inconsistencies in experimental approach (Requena et al. 2005, McNaughton, Siegler, and Midgley 2008). Nevertheless, when key methodological components such as dosage (0.3 $g.kg^{-1}$; McNaughton 1992a, Requena et al. 2005), timing of ingestion (~ 60/90 mins pre-

exercise, Renfree 2007, Price and Singh 2008) and exercise protocol (constant load exercise to exhaustion; Matson and Tran 1993, Hopkins et al. 2001) are adopted ergogenic benefit is more likely to be observed (Matson and Tran 1993).

In an attempt to elucidate the effects of modulating acid-base balance at a tissue level several studies have examined the effects of metabolic acidosis (ACD) and alkalosis (ALK) on isolated muscle performance. Spriet et al. (1985) induced ACD by lowering the [HCO_3^-] in the isolated muscle perfusate from ~ 24mM to ~ 13 mM. This significantly increased the rate of muscle tension decay and reduced absolute muscle tension in the gastrocnemius-plantaris-soleus muscle group, during fatiguing isometric stimulation, when compared to CON. Conversely, Spriet et al. (1986) found that inducing ALK by increasing the [HCO_3^-] from ~ 21 mM to ~ 27 mM had no effect on peak isometric tension or tension decay compared to CON. Furthermore, Broch-Lips et al. (2007) examined the effect of 40 mM and 25 mM [HCO_3^-] on isometric force production in isolated rat skeletal muscle. The elevated [HCO_3^-] had no significant effect on force maintenance during continuous stimulation or recovery of force during brief tetanic stimulation in either SOL or EDL muscles at 30°C. Similarly, 40 mM of HCO_3^- had no significant effect on isometric force maintenance during either continuous stimulation or intermittent stimulation protocols (1 s on; 3 s off) at 37°C in SOL. No significant effects were also observed on force production in EDL muscle (Broch-Lips et al. 2007).

Although the studies noted have examined the effects of high and low [HCO_3^-] on muscle performance the current body of *in vitro* isolated muscle research has a number of methodological concerns. This is particularly so when trying to apply the results to *in vivo* human muscle performance. For example, no research to date has used concentrations of HCO_3^- that are typically achieved in the blood of human participants (~ 32 mM; Kolkhorst et al. 2004, Price and Singh 2008; Lindh et al. 2008; Siegler et al. 2010) following the recommended supplementation dosage (0.3 $g.kg^{-1}$; McNaughton 1992, Requena et al.

2005). Moreover, during mammalian locomotion muscles that are attached to moving skeletal structures, either directly or indirectly, undergo repetitive length changes (Josephson 1993). Approximation of such length changes *in vitro* facilitates the evaluation of important components of exercise performance such as tolerance to the effects of fatigue in mammalian muscle (James, Wilson, and Askew 2004). Unfortunately, previous research that has used elevated [HCO_3^-] and simulated *in vivo* exercise performance used isometric muscle contractions (Broch-Lips et al. 2007). Unlike the work loop method, isometric contractions do not consider maximal force production in terms of any interaction between activation and relaxation time and/or any passive resistance to muscle lengthening that occurs during cyclical length changes during mammalian locomotion.

To the best of our knowledge, no research has examined the effects of $NaHCO_3$ on isolated mammalian muscle during such cyclical length changes. Therefore, the aim of this study was to investigate the efficacy of elevated buffer capacity ([HCO_3^-]) through administration of $NaHCO_3$, on maximally stimulated isolated mouse soleus (SOL) and extensor digitorum longus (EDL) muscles undergoing cyclical length changes at physiological temperature (37°C). The first objective was to evaluate the acute effects of $NaHCO_3$ on isolated muscle maximal PO and the second objective was to evaluate the effects of $NaHCO_3$ on isolated muscle fatigued at the intensity that evoked maximal PO. Both objectives were achieved using the work loop technique which has previously been used to evaluate the direct effects of other nutritional ergogenic aids on muscle performance (James, Wilson, and Askew 2004, James et al., 2005, Tallis et al. 2012).

6.3 Methods

6.3.1 Dissection

Eight-to-ten week old female white mice (strain CD1, Charles River, UK) were bred and kept at the University. The mice were weighed (body mass: 31.9 ± 1.9 g, n = 24) and

then killed by cervical dislocation in accordance with the British Home Office Animals (Scientific Procedures) Act 1986, Schedule 1. The use of animals in this study was approved by the University Ethics Committee.

For the acute protocol a different muscle was taken from each hind limb of each mouse (e.g. SOL: right hind limb; EDL: left hind limb) alternating with each animal. This process was undertaken to counterbalance any effects of anatomical dominance that may exist in this cohort of animals, with each individual muscle acting as its own control. For the fatigue protocol the same muscle was taken from each limb of the *same* animal so that muscles could be directly compared between treatments ($NaHCO_3$ vs. CON).

In total, 48 muscles were evaluated from 24 mice (objective 1, acute muscle performance: SOL n=8, EDL n=8; objective 2, fatigue resistance: SOL n=16 and EDL: n=16 (8 each for $NaHCO_3$ and CON). Throughout the dissection procedure the muscle preparation was maintained in refrigerated and oxygenated (95% O_2; 5% CO_2) Krebs-Henseleit solution (NaCl 118mM; KCl 4.75mM; $MgSO_4$ 1.18 mM; $NaHCO_3$ 25 mM; KH_2PO_4 1.18 mM; glucose 10 mM; $CaCl_2$ 2.54 mM; pH 7.59 ± 0.03 at room temperature prior to oxygenation and pH 7.46 ± 0.03 at experimental temperature (37°C) after oxygenation).

Soleus (SOL) and extensor digitorum longus (EDL) muscles were isolated from the hind limbs and then pinned out at approximately their resting length at laboratory temperature (24-25^{o}C). For each preparation the tendon and a small piece of bone was left attached at the proximal and distal ends. Aluminium foil T-clips were wrapped around each tendon, leaving the bone at the back of the clip, to prevent tendon slippage during muscle activation (James et al. 2005).

6.3.2 Experimental set-up

Each muscle preparation was attached via the foil clips to a force transducer (UF1, Pioden Controls Ltd., Kent, UK) and a displacement transducer (V201, Ling Dynamic Systems, Hertfordshire, UK) at opposing ends in one of two identical set-ups. Position of the motor arm was detected using a Linear Variable Displacement Transformer (LVDT; DFG5.0, Solartron Metrology, Sussex, UK). Throughout the experimental procedure the muscle was maintained at standard physiological temperature (37 ± 0.2^{o}C; Askew and Marsh 1997) in circulating oxygenated Krebs-Henseleit solution. The preparation was stimulated via parallel platinum electrodes whilst held at constant length to generate a series of isometric twitches. The electrodes were not in contact with the nerve branch or the fibre itself but stimulated the muscle via the surrounding fluid.

6.3.3 Isometric protocol

Muscle length and stimulus amplitude (12-16V for SOL; 14-18V for EDL) were optimised in order to achieve maximal isometric twitch force. The muscle length that corresponded to maximal isometric twitch force was measured using an eyepiece graticule fitted to a microscope, and was defined as L_0. Mean muscle fibre length was calculated as 85% of L_0 for SOL and 75% of L_0 for EDL (James, Altringham, and Goldspink 1995, James, Wilson, and Askew 2004). Maximal isometric tetanic force was also measured by subjecting the preparation to a burst of electrical stimuli (350 ms for SOL; 250 ms for EDL; James, Wilson, and Askew 2004, James et al. 2005). Stimulation frequency was optimised to yield maximal tetanic force (140 Hz for SOL; 200 Hz for EDL; Askew, Young, and Altringham 1997, James, Altringham, and Goldspink 1995, James, Wilson, and Askew 2004). After each tetanic stimulation a 5 minutes rest period was imposed to ensure that the muscle recovered fully. Upon completion of the final 5 minute rest period, each preparation was subjected to the following work loop protocol.

6.3.4 Work loop protocol

The work loop technique assesses the power output of isolated muscle whilst undergoing cyclical length changes (Josephson 1985; James et al. 2005). Starting at L_0, each muscle was subjected to four sinusoidal length change cycles per set, at a total symmetrical strain of 0.10 (10% of L_0). More simply the muscle was lengthened by 5% from L_0, followed by a shortening to 5% shorter than L_0, before returning to L_0 (i.e. 100% to 105% to 95% to 100%). This was repeated 4 times per set. Each complete sinusoidal length change yields one work loop. A strain of 10% was used as it yields maximum power *in vitro* at the cycle frequencies used in the present study (James, Altringham, and Goldspink 1995).

Length changes were delivered at a cycle frequency of 5Hz for SOL and 8Hz for EDL. For SOL the 5Hz cycle frequency represents that what elicits maximal power output *in vitro* and is attainable *in vivo* (James, Altringham, and Goldspink 1995, Askew and Marsh 1997, Askew, Young, and Altringham 1997). Although 10-12Hz represents the range of cycle frequencies that elicit maximal power for EDL muscle, mice are unable to attain a stride frequency much greater than 8Hz (James, Altringham, and Goldspink 1995).

Muscle stimulation and length changes were controlled using custom written software (Testpoint, CEC, Massachusetts, USA) via a D/A board (KPCI3108, Keithley Instruments, Ohio, USA). Data were sampled at a rate of 10 kHz and a work loop was formed by plotting force against length. The area of a work loop represents the net work done by the muscle during a single length change cycle (Josephson 1985). The muscles were electrically stimulated and the stimulus burst duration (i.e. total duration of stimulation in each length change cycle) was altered until maximal net power output was achieved. The burst duration that achieved maximal power output was typically 60 ms for SOL and 59 ms for EDL. These values are similar to those found in previous studies using the work loop technique (James, Wilson, and Askew 2004, Vassilakos et al. 2009). Stimulation phase shifts (i.e. the time at which stimulation commenced, stated with respect to the time that the muscle reached maximum length; such that a negative value indicates that stimulation

started before maximal muscle length was reached) of -10 ms and -2 ms were used for SOL and EDL respectively (Tallis et al. 2012). The second loop of each set of four work loops was used as an indicative measure of performance for each trial (James et al. 2005, Tallis et al. 2012). A 10 minute rest was given between each set of work loop trials to ensure sufficient recovery (James, Wilson, and Askew 2004).

6.3.5 Treatment solutions

The composition of the control (CON; standard Krebs- Henseleit) solution was: NaCl 118mM; KCl 4.75mM; $MgSO_4$ 1.18 mM; $NaHCO_3$ 25 mM; KH_2PO_4 1.18 mM; glucose 10 mM; $CaCl_2$ 2.54 mM; pH 7.59 ± 0.03 at room temperature prior to oxygenation and pH 7.46 ± 0.03 at experimental temperature (37°C) after oxygenation. The composition of the experimental ($NaHCO_3$) solution was: NaCl 111 mM; KCl 4.75 mM; $MgSO_4$ 1.18 mM; $NaHCO_3$ 32 mM; KH_2PO_4 1.18 mM; glucose 10 mM; $CaCl_2$ 2.54 mM; pH 7.74 ± 0.02 at room temperature prior to oxygenation and pH 7.56 ± 0.03 at experimental temperature (37°C) after oxygenation. The experimental solution was designed to replicate the concentration of $NaHCO_3$ achieved in human blood after oral supplementation (~ 32 mM [HCO_3^-], Price and Singh 2008, Lindh et al. 2008, Cameron et al. 2010, Siegler et al. 2010) and was equimolar in Na^+ content to CON.

6.3.6 Objective 1: Acute protocol - the effects of $NaHCO_3$ on maximal power output

Muscle preparations were subjected to sets of 4 work loops at 10 minute intervals over a period of 130 minutes. The acute protocol consisted of 3 sets of 4 work loops as control measurements in standard Krebs-Henseleit solution (Pre-CON), followed by 6 sets of 4 work loops as treatment measurements in either the CON or $NaHCO_3$ solutions (i.e. the treatment intervention) and 4 final sets of 4 work loops as control measurements in the CON solution (Post-CON, washout).

6.3.7 Objective 2: Fatigue protocol - the effects of $NaHCO_3$ on skeletal muscle fatigue

The fatigue protocol consisted of 3 sets of 4 work loops as control measurements in standard Krebs-Henseleit solution (Pre-CON), followed by 3 sets of 4 work loops as treatment measurements in either the CON or $NaHCO_3$ solutions (i.e. the treatment intervention). Data from the acute protocol indicated that 3 sets of work loops allowed the muscle to become fully accustomed to the treatment intervention. Ten minutes following the last treatment measurement the muscles were subjected to a fatigue run consisting of 100 consecutive work loops (12.5 s duration at 0.125 s (8Hz length change cycle frequency) per work loop for EDL; 20 s duration at 0.20 s (5Hz length change cycle frequency) per work loop for SOL). Ten minutes after the fatigue protocol was completed (i.e. rest period) muscle preparations were then subjected to 6 further sets of 4 work loops at 10 minute intervals over a period of 60 minutes in standard Krebs-Henseleit solution (post-CON). These post-fatigue measurements were conducted to assess the rate of recovery of muscle function after the experimental intervention and to verify that decreases in performance during the fatigue protocol were due to fatigue and not death of muscle fibres.

6.3.8 Muscle mass and dimension calculations

At the end of the experiment the muscle was disconnected from the apparatus and the tendons and bones removed leaving the muscle intact. The muscle was blotted on tissue paper to remove excess fluid and then placed on an electronic balance (Mettler Toledo B204-S, Zurich, Switzerland) to determine the wet muscle mass. All muscles were analysed for muscle quality (pre-treatment isometric stress (i.e. twitch/tetanus) [force normalised to muscle cross-sectional area] and normalised (mass specific) power output. Mean muscle cross-sectional area was calculated from mean fibre length, muscle mass and an assumed muscle density of 1060 kg m^{-3} (Méndez and Keys 1960). Isometric stress was calculated as

force divided by mean muscle cross-sectional area. Muscle power output was normalised to muscle mass to express power as $W.kg^{-1}$.

6.3.9 Power output analysis

In humans PO decreases to ~ 60% of maximum during sprint running of ~ 20 s (Cheetham et al. 1986) which most closely resembles the protocol used in this study. Therefore analysis of PO during the fatigue run was analysed until the muscle had decreased to 60% of maximum PO (T_{LIM60}) for both EDL and SOL.

In the absence of vascular perfusion, isolated muscle PO may reduce over time due to the progressive development of an anoxic core (Barclay 2005). In order to avoid deterioration in muscle performance masking the effects of $NaHCO_3$ on acute PO a first order regression equation was calculated using the control data and washout data to identify the linear relationship between muscle power output and time (Tallis et al. 2012). In the present study power output of muscle preparations typically decreased by 12% (EDL) and 4% (SOL) between pre-CON and post-CON control measurements. Therefore, the regression equation was used to determine theoretical control muscle power output for each time point during $NaHCO_3$ treatment to allow performance during treatment to be compared with the theoretical control performance of the muscle being tested (Tallis et al. 2012).

6.3.10 Statistical analysis

Statistical analysis was completed using PASW (SPSS; v17, Chicago, USA) and Excel (Microsoft; v2007, Redmond, USA). For all analyses, assumptions for parametric tests were carried out as per section 3.8. Based on this initial analysis a number of different parametric (independent and paired t-tests, single factor repeated measures ANOVA) and non-parametric (Mann-Whitney U, Wilcoxon, Friedman) tests were utilised. Statistical

significance was accepted at $P < 0.05$. For comparisons of muscle quality (muscle stress and mass-specific power output) before treatment, between groups (i.e. acute treatment vs. fatigue treatment vs. fatigue CON), where significance was achieved using single factor ANOVA, pairwise comparisons (least significant difference; LSD) were undertaken. LSD comparisons were chosen as they are the most powerful when analysing 3 levels/groups (Howell 2007). Figures quoted are mean values ± standard deviation (SD) unless otherwise stated. For ANOVA effect sizes (ES) are reported as the partial η^2 value. Elsewhere the effect size was calculated as the mean difference divided by the root mean squared standard deviation.

6.4 Results

6.4.1 Muscle quality

There was no significant difference in the tetanic stress (SOL: $P = 0.82$; EDL: $P = 0.44$) or twitch stress (SOL: $P = 0.47$; EDL: $P = 0.75$), before treatment, between any of the treatment groups (i.e. acute treatment vs. fatigue treatment vs. fatigue CON muscles). Similarly, there was no significant difference in [pre-treatment] mass-specific PO for EDL muscles ($P = 0.22$; i.e. acute treatment vs. fatigue treatment vs. fatigue CON muscles). In contrast there was a significant difference ($P = 0.049$) between the mass-specific PO of the SOL muscles used in the acute protocol compared to SOL muscles used in the $NaHCO_3$ condition during fatigue (post-hoc LSD: $P = 0.02$). However, as these muscles are not directly compared this has minimal impact on the results. Most importantly, there was no significant difference in the pre-treatment mass-specific PO between SOL muscles subsequently used in $NaHCO_3$ and CON fatigue trials (i.e. muscles from the same animal; post hoc LSD $P = 0.33$). Therefore, any differences between experimental trials during fatigue were due to the applied treatment. Table 6.1 outlines twitch and tetanus values and mass-specific work loop PO for SOL and EDL.

Table 6.1 Isometric stress and work loop power output for SOL and EDL muscles before treatment (Data represented as mean ± SD; n=24 for each muscle type)

	SOL	EDL
Twitch Stress (kN m^2)	39 ± 12	36 ± 12
Tetanus Stress (kN m^2)	227 ± 59	154 ± 46
Power Output (W.kg^{-1})	38 ± 11	65 ± 18

6.4.2 The acute effects of $NaHCO_3$ on maximally stimulated EDL and SOL muscles

There was no difference in PO for EDL and SOL between pre and post treatment CON work loops (P = 0.55, P = 0.53, respectively) demonstrating consistency of performance either side of treatment (Figure 6.1). Therefore, all CON results were pooled and compared against the PO achieved during the $NaHCO_3$ treatment (Tallis et al. 2012).

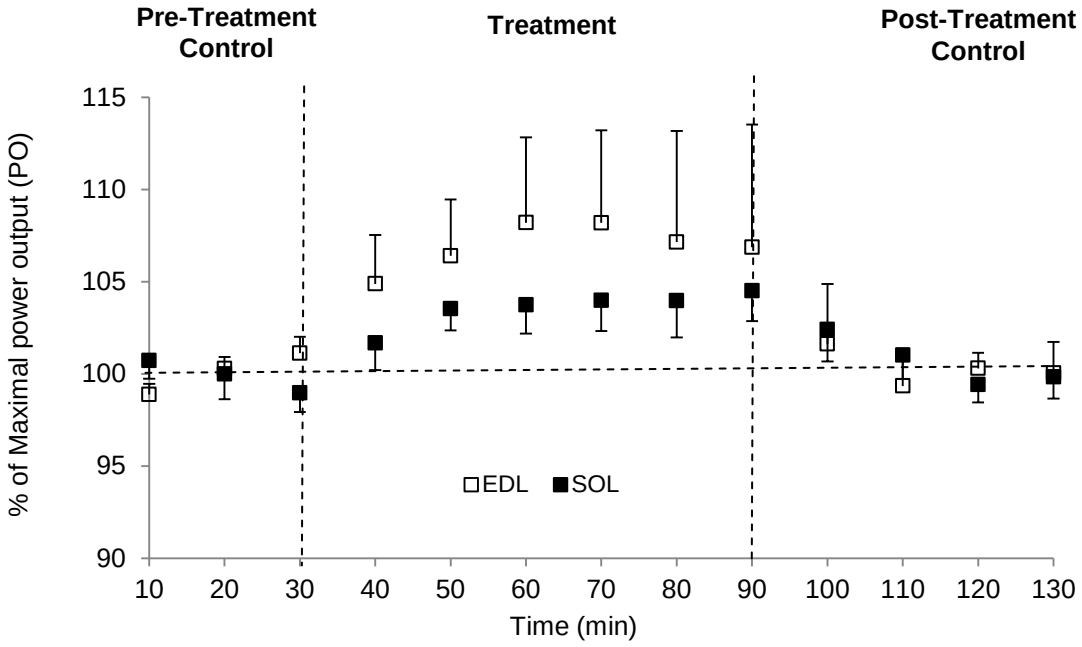

Figure 6.1 The acute effects of $NaHCO_3$ on maximally stimulated mouse soleus (SOL) and EDL muscles. Vertical dotted lines represent timings of the start and end of the treatment (n=8 for each muscle; Values are displayed as a percentage of theoretical control work loop power output [horizontal dotted line] and data represented as mean ± SD).

Power output was on average 7.0 ± 4.8 % greater for $NaHCO_3$ treated EDL muscles ($P < 0.001$; ES = 2.0) and 3.6 ± 1.8 % greater for $NaHCO_3$ treated SOL muscles ($P < 0.001$; ES = 2.3) compared to CON (Figure 6.1). The acute effects of $NaHCO_3$ on EDL were significantly greater ($P < 0.001$; ES = 0.9) than on SOL (Figure 6.1). The increase in acute PO as observed in Figure 6.1 was due to an increase in the force generated during shortening (Figure 6.2).

6.4.3 The effects of $NaHCO_3$ on fatigue of maximally stimulated EDL and SOL muscles

6.4.3.i Extensor digitorum longus (EDL)

At a group level there was no significant difference between treatments in the time taken for EDL power output to reduce to 60% of maximum (T_{LIM60}; CON: 2.55 ± 0.32 s; work loop 20 ± 3, $NaHCO_3$: 2.36 ± 0.27 s; work loop 19 ± 2, $P = 0.21$; ES = 0.6) i.e. no significant difference in the pattern of fatigue. However, of the 8 paired muscles, % PO at T_{LIM60} was greater in CON on 4 occasions, twice for $NaHCO_3$ with the remaining two pairs equal thus demonstrating marked inter-individual variation. The mean % of maximal control PO (i.e. PO recorded at each time point [every second work loop] for each muscle was pooled) produced by EDL until T_{LIM60} was not different between CON and $NaHCO_3$ (87 ± 14% and 87 ± 14% respectively, $P = 0.97$) but significantly reduced over time ($P < 0.001$; Figure 6.3). However, as shown in Figure 6.1, $NaHCO_3$ increased initial PO by ~ 7% in EDL, so PO would be higher in $NaHCO_3$ than in CON for the initial section of the fatigue protocol shown in Figure 6.3.

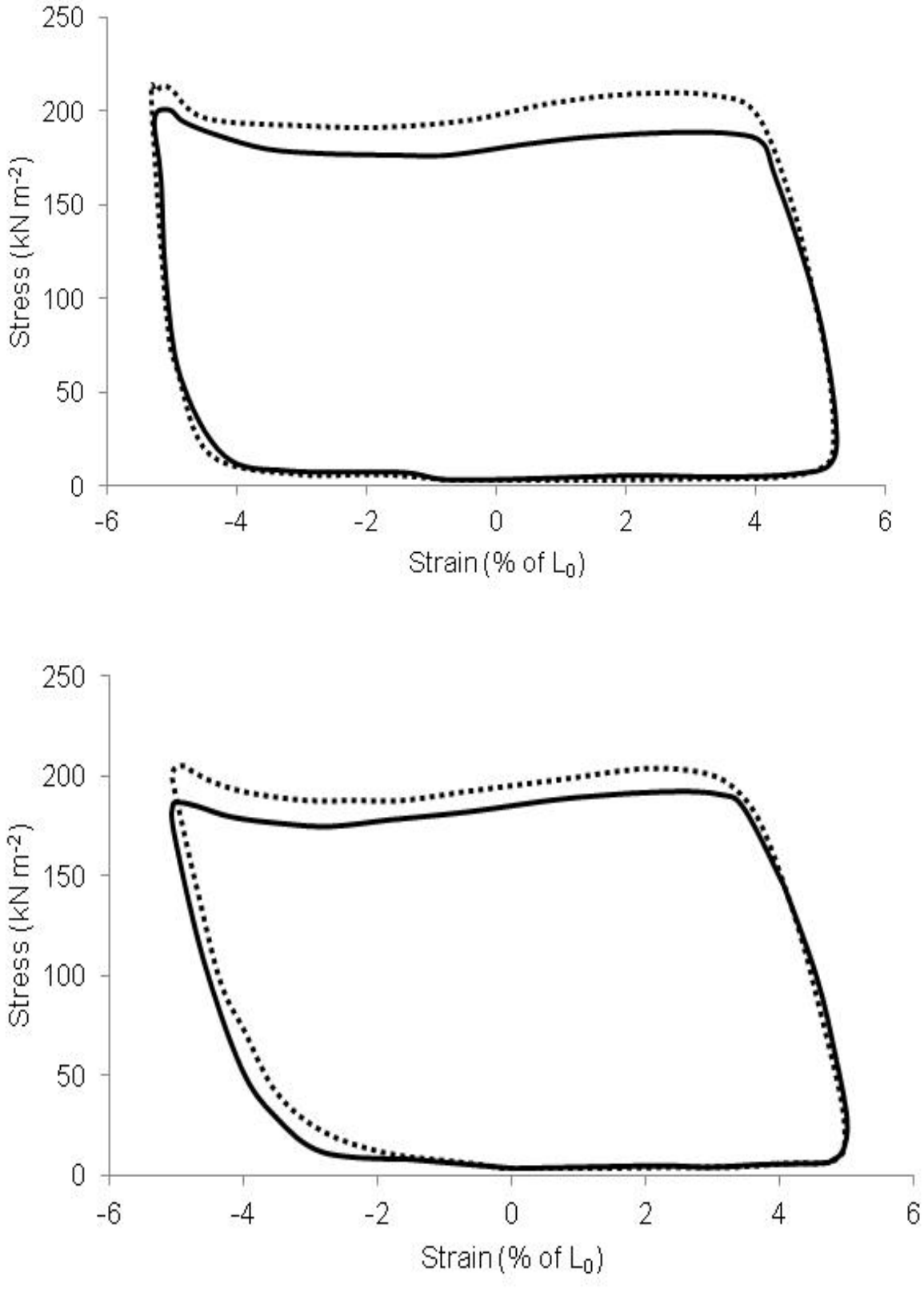

Figure 6.2 Typical work loop shapes for EDL (top) and SOL (bottom) during acute protocol. Dotted lines represent $NaHCO_3$ treatment and full lines represent CON.

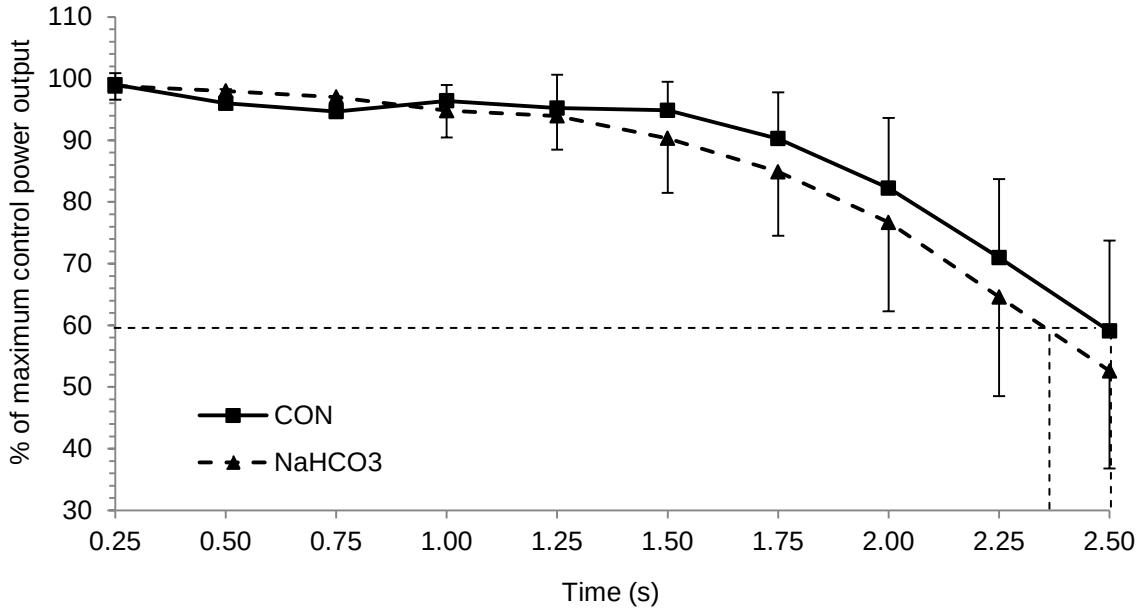

Figure 6.3 The effects of $NaHCO_3$ on T_{LIM60} in maximally stimulated mouse EDL muscle (n=8 per treatment. Values are displayed as a % of maximal control work loop PO ± SD. Each 0.25 s represents 2 work loops, i.e. values are only shown for every other work loop).

6.4.3.ii Soleus (SOL)

At a group level there was no significant difference between treatments for T_{LIM60} for SOL ($NaHCO_3$: 5.58 ± 0.79 s; work loop 28 ± 4, CON: 4.93 ± 0.73 s; work loop 25 ± 4, P = 0.18; ES = 0.9) i.e. no significant difference in the pattern of fatigue. However, of the 8 paired muscles, % PO at T_{LIM60} was greater in $NaHCO_3$ on 5 occasions and on 3 occasions for CON which similar to EDL demonstrates marked inter-individual variation. The mean % of maximal control PO produced by SOL until T_{LIM60} was not different between CON and $NaHCO_3$ (84 ± 14% and 85 ± 12%, respectively, P = 0.47) but significantly reduced over time (P < 0.001; ES = 0.9; Figure 6.4). However, as shown in Figure 6.1, $NaHCO_3$ increased initial PO by 3.6% in SOL, so mass specific power output would be higher in $NaHCO_3$ than in CON for the initial section of the fatigue protocol shown in Figure 6.4.

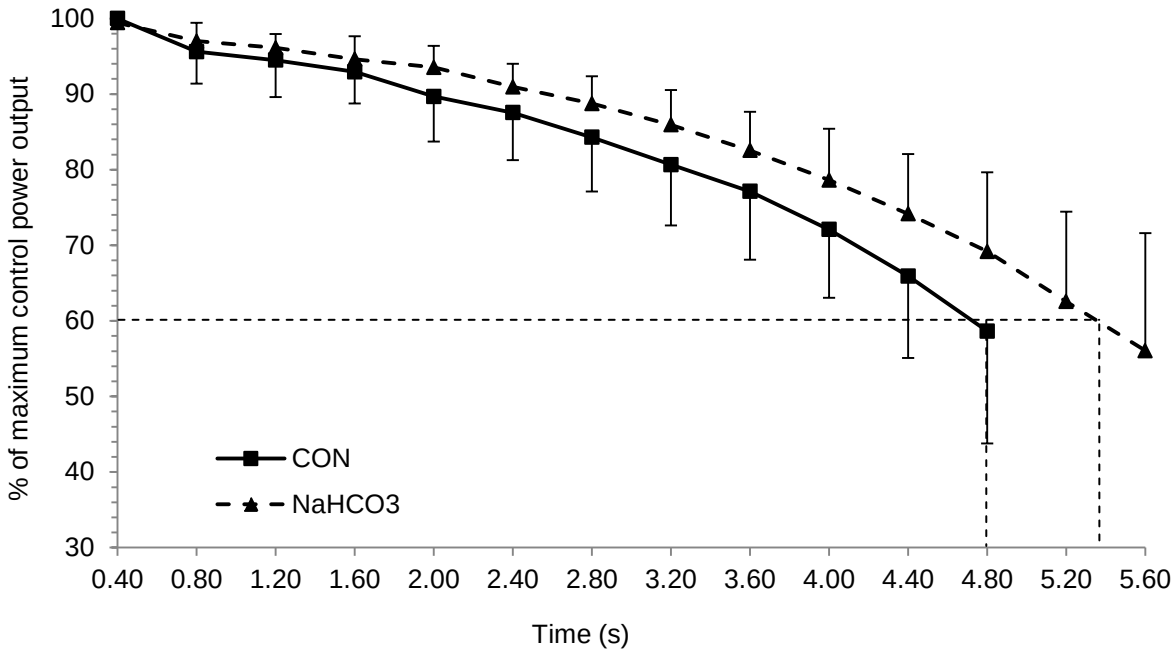

Figure 6.4 The effects of $NaHCO_3$ on T_{LIM60} in maximally stimulated mouse SOL muscle (n=8 per treatment. Values are displayed as a % of maximal control work loop PO ± SD. Each 0.40 s represents 2 work loops, i.e. values are only shown for every other work loop).

6.4.4 Rate of recovery of PO after fatigue of maximally stimulated EDL and SOL muscles

6.4.4.i Extensor digitorum longus (EDL)

There was a significant difference between treatments in the recovery of PO for EDL (P = 0.04, ES = 0.2). Over the sixty minute recovery period CON demonstrated a greater mean recovery (62 ± 25 %) than $NaHCO_3$ (56 ± 27 %). Individual comparisons (i.e. treatment * time) revealed that recovery of PO was greater for CON (35 ± 11 %) compared to $NaHCO_3$ (24 ± 12 %) after 10 minutes (P = 0.03; Figure 6.5). Recovery of PO for EDL post muscle fatigue increased over time (P < 0.001; ES = 0.8) with both CON (84 ± 34 %) and $NaHCO_3$ (78 ± 18 %) peaking in absolute terms after sixty minutes recovery. Overall, the combined mean PO after 50 minutes recovery was 73 ± 22 % which did not increase further (post hoc LSD: P = 0.13).

6.4.4.ii Soleus (SOL)

In contrast to EDL, there was no significant difference in recovery between treatments (P = 0.19). Overall, after 60 minutes of recovery $NaHCO_3$ demonstrated a mean recovery of 92 ± 7% of control maximal PO compared with 90 ± 9% for CON (Figure 6.5). Recovery of PO for SOL post muscle fatigue increased over time (P < 0.001; ES = 0.4) with both CON (93 ± 7 %) and $NaHCO_3$ (96 ± 9 %) peaking after twenty minutes recovery. Overall, the combined mean PO after 20 minutes recovery was 94 ± 8 % which did not significantly increase further during recovery (post hoc LSD: P > 0.13 in all cases).

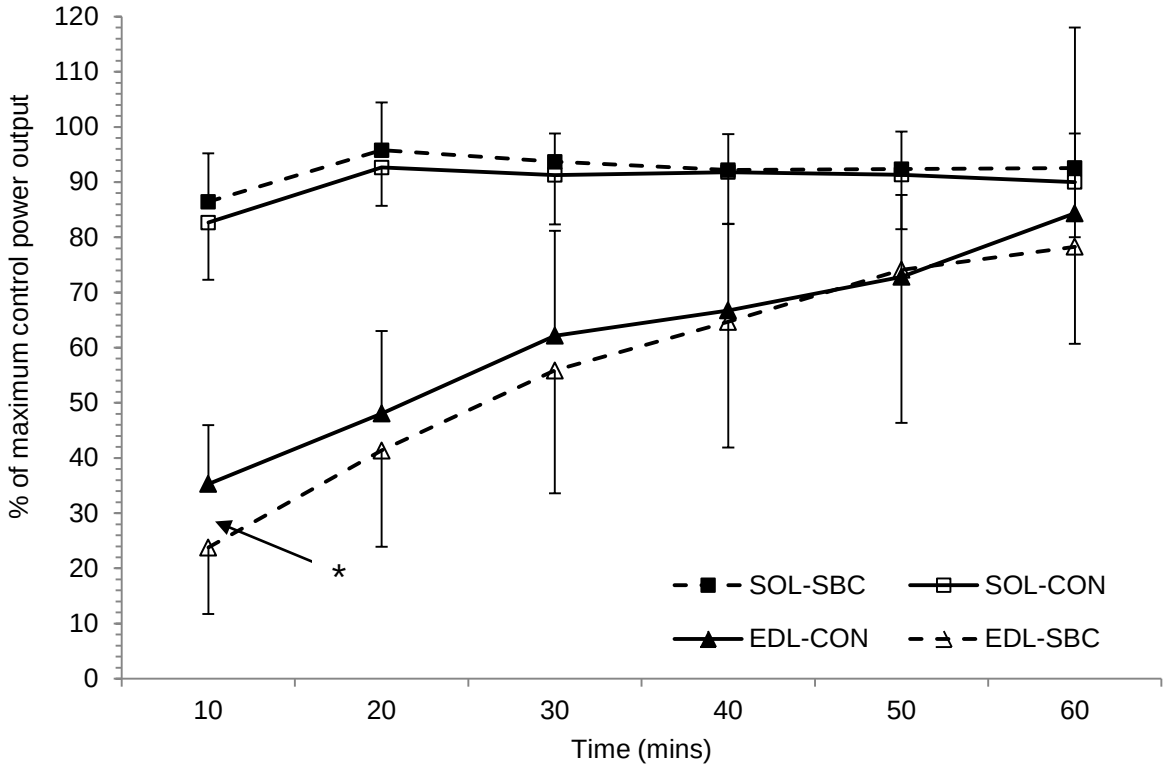

Figure 6.5 The effects of $NaHCO_3$ on recovery after fatigue in maximally stimulated mouse SOL and EDL muscles (n=8 per treatment. Values are displayed as a % of maximal work loop PO ± SD).* P = 0.03.

Recovery of mean maximum control power output for combined data was significantly greater (P < 0.001; ES = 1.7) in SOL (91 ± 8%) than EDL (59 ± 26 %; Figure

6.5). Importantly, both EDL (~ 80%) and SOL (~ 90%) muscles recovered almost completely within one hour of the fatigue run demonstrating that fatigue, rather than damage, was the overwhelming reason for performance decreases during the fatigue protocol.

6.6 Discussion

6.6.1 Muscle quality

The mean SOL tetanic stress of 227 kN m^{-2} measured in the present study is slightly higher, but similar to, a range of 189 to 224 kN m^{-2} of previously published values, measured in mouse SOL muscle at a similar temperature (Brooks and Faulkner 1988, James, Altringham, and Goldspink 1995, Askew, Young, and Altringham 1997, Tallis et al. 2012). Likewise the mean SOL mass specific power output of 38 $W.kg^{-1}$ measured in the present study is slightly higher than previously published values that range from 31.7 to 34 $W.kg^{-1}$ (James, Altringham, and Goldspink 1995, Askew, Young, and Altringham 1997, Tallis et al 2012). The mean EDL tetanic stress of 154 kN m^{-2} measured in the present study is relatively low when compared to previously published values that range from 230 to 243 kN m^{-2} (Brooks and Faulkner 1988, James, Altringham, and Goldspink 1995, Askew and Marsh 1997). Likewise the mean EDL mass specific power output of 65 $W.kg^{-1}$ measured in the present study is relatively low when compared to the previously published value of 98 $W.kg^{-1}$ measured under similar conditions (James, Altringham, and Goldspink 1995). As the stability of the muscle preparations over time in this study was comparable to previous studies it seems unlikely that the lower EDL stress value is due to muscle damage. Therefore, the variation in muscle stress and mass specific power between studies would most likely be due to differences in muscle fibre type and morphology between the strains and ages of mice used in different studies.

6.6.2 The acute effects of $NaHCO_3$ on maximally stimulated EDL and SOL muscles

In the present study, isolated mouse muscles incubated at 37°C in modified Krebs-Henseleit solution (~ 32 mM [HCO_3^-]) generated greater acute power output (EDL = 7.0 ± 4.8 %; SOL = 3.6 ± 1.8 %; Figure 6.1) during cyclical contractions than CON muscles incubated in standard Krebs-Henseleit solution (~ 25 mM [HCO_3^-]). By analysing typical work loop shapes (Figure 6.2) we have demonstrated that the increase in power output is due to increased force generation during shortening. This is the first study to demonstrate such novel findings. In contrast, Broch-Lips et al. (2007) found no significant difference in isometric force production in the presence of a greater [HCO_3^-] (~ 40 mM [HCO_3^-]) in both SOL and EDL rat muscles. However, Broch-Lips et al. (2007) used isometric contractions whereas the work-loop technique used within the present study subjects the isolated muscle to activation and length change patterns that better approximate *in vivo* locomotory function (Josephson 1985). By approximating the type of dynamic muscle activities that are likely to occur in many sporting activities, the results of the present study give the best indication to date that $NaHCO_3$ has direct effects on acute skeletal muscle performance in mammals, by means of augmented force production during shortening. Such data provides important supporting mechanistic evidence for the acute ergogenic effect observed in humans (McNaughton 1992a, McNaughton, Ford, and Newbold 1997)

6.6.3 The effects of $NaHCO_3$ on time to fatigue of maximally stimulated EDL and SOL muscles

At the group level treatment of EDL and SOL muscles with sodium bicarbonate solution (~ 32mM [HCO_3^-]) did not enhance T_{LIM60} compared to CON. Moreover, the % of maximal control PO produced by EDL and SOL was not different between treatments (Figures 6.3 and 6.4). However, muscle performance varied quite considerably. Of the 8 paired EDL muscles T_{LIM60} was greater in CON on 4 occasions, twice for $NaHCO_3$ with the remaining two pairs equal. Of the 8 paired SOL muscles T_{LIM60} was greater in $NaHCO_3$ on 5 occasions and on 3 occasions for CON. Such individual variability in the direct fatigability of

skeletal muscle might contribute to the equivocal results seen during *in vivo* research in humans (Requena et al. 2005, McNaughton, Siegler, and Midgley 2008, Price and Simons 2010, studies 2 and 4, chapters 5 and 7, respectively). Furthermore, it should also be noted that due to the acute effects of $NaHCO_3$ (Figures 6.1 and 6.2) this would cause initial power (and force) to be higher such that in absolute terms $NaHCO_3$ treated muscles would be producing higher power for longer.

The lack of ergogenic benefit in whole group data is similar to those of Broch-Lips et al. (2007) who demonstrated no ergogenic effect, using a treatment of ~ 40 mM [HCO_3^-], on either SOL or EDL during ~ 3 to 5 mins continual isometric tetanic stimulations of isolated rat muscle. The protocol adopted by Broch-Lips et al. (2007) was used to mimic the 1-7 minute window in which $NaHCO_3$ is deemed to be most effective (Linderman and Fahey 1991; Linderman and Gosselink 1994, Broch-Lips et al. 2007). The lack of ergogenic effect was noted despite a considerably greater [HCO_3^-] than used in the present study (40 mM vs. 32 mM, respectively) and a concomitant higher pH (7.60 vs. 7.56, respectively). Similarly, Lindinger et al. (1990) demonstrated that increasing the perfusate concentration to ~ 29 mM [HCO_3^-] (by additional $NaHCO_3$) had no effect on force production compared to CON (~ 21 mM [HCO_3^-]) during intense isometric stimulation in isolated rat hindlimb.

6.6.4 Practical implications of acute power output results

The improvements of acute power output in the present study (EDL = 7.0 ± 4.8 %, SOL = 3.6 ± 1.8 %) are similar in magnitude to previous research in humans which demonstrated that 0.3 $g.kg^{-1}$ $NaHCO_3$ (~32 mM [HCO_3^-]) exhibited mean increases in peak power of 10.8% (McNaughton 1992) and 5.7% (McNaughton, Ford, and Newbold 1997) compared to CON and PLA during 60 s maximal cycling. The present study suggests that increases in PO in mammals appear to be due to greater force development throughout muscle shortening. Therefore, the results of the present study give the best indication to date

that $NaHCO_3$ has direct peripheral effects on the acute power output of both fast and slow twitch skeletal muscle. This has important training and performance implications for those individuals undertaking high-intensity physical activity of ~ 60 seconds where enhanced acute power output is likely to contribute to performance improvement. Indeed, supplementation with $NaHCO_3$ during training may result in positive physiological adaptations beyond training alone. Thomas et al. (2007) demonstrated that regular short term high-intensity exercise in rats (5 sessions per week for 5 weeks) conducted with $NaHCO_3$ supplementation resulted in significantly larger increases in monocarboxylate transporter 4 (MCT4) abundance and citrate synthase activity than PLA (training) and CON. Interestingly, this was only observed in SOL with no changes in EDL. Similarly, Bishop et al. (2010) demonstrated that 5 weeks of interval training in rats conducted with $NaHCO_3$ supplementation was associated with greater improvements in both mitochondrial mass and mitochondrial respiration in SOL than PLA (training) and CON. This contributed to a 52% increase in time to exhaustion (T_{LIM}) compared to PLA. Furthermore, Edge, Bishop, and Goodman (2006) demonstrated that high-intensity training in humans was associated with greater improvement in T_{LIM} (164 vs. 123%) and lactate threshold (26 vs. 15%) with $NaHCO_3$ supplementation compared to PLA. Therefore, using $NaHCO_3$ may promote both enhancements in acute power output and/or positive muscle specific physiological adaptations that are likely to enhance both acute and endurance performance. Interestingly, no research has previously examined the efficacy of $NaHCO_3$ on physical performance pre and post-training, something we have addressed in study 4 (chapter 7).

6.6.5 Practical implications of fatigue results

Treatment of EDL and SOL muscles with sodium bicarbonate solution (~ 32 mM [HCO_3^-]) did not enhance T_{LIM60} compared to CON. Moreover, the percentage of maximal control PO produced by EDL and SOL was not different between treatments. However, individual muscle performance varied quite considerably. For example there was a trend for

T_{LIM60} in the EDL CON trial to be 8% longer than $NaHCO_3$ (i.e. EDL fatigued faster during treatment than CON). Of the 8 paired EDL muscles T_{LIM60} was greater in CON than $NaHCO_3$ on 4 occasions (50%) with two pairs equal. In contrast the trend for SOL at T_{LIM60} was 13% greater for $NaHCO_3$ than CON. Of the 8 paired SOL muscles T_{LIM60} was greater in $NaHCO_3$ on 5 occasions (63%). Such variability of performance with $NaHCO_3$ has been previously reported in humans during T_{LIM} trials in both running (Price and Simons 2010) and cycling (Saunders et al. 2011, studies 2 and 4, chapters 5 and 7, respectively). This led the authors to suggest that humans are either 'responders' or 'non-responders' to $NaHCO_3$ supplementation. The results of the present study suggest that such a classification might be appropriate at a skeletal muscle level.

6.6.6 Muscle fibre distribution

In terms of application to human performance the final key practical implication based on the results of the present study relates to muscle fibre type distribution. As noted previously $NaHCO_3$ facilitated increases in acute PO for both EDL and SOL. However, the benefit observed in EDL was ~ 100% greater than in SOL (7.0 % vs. 3.6%). Therefore, humans with a greater distribution of (predominantly) type II fibres (FT), such as in EDL, may be more likely to see ergogenic benefit with $NaHCO_3$ during high-intensity exercise of short duration (i.e. ~ 60 seconds) where the ability to produce high acute PO is likely to be important to performance. Although ergogenic benefit was also observed in SOL, those individuals who have a greater percentage of type I fibres (ST) such as in SOL are likely to see less ergogenic benefit during high-intensity exercise of short duration (i.e. ~ 60 seconds). Therefore, whilst having an appropriate morphology for a particular sporting event is nothing new the *in vitro* results of the present study suggest that different muscle fibre types respond in different ways to $NaHCO_3$ supplementation. As such, an individual's overall muscular fibre type distribution might not only impact on their athletic performance but also the extent to which $NaHCO_3$ might facilitate further performance improvement.

The individual variation in muscular distribution raises implications for researchers when recruiting human volunteers for studies evaluating $NaHCO_3$. Often the homogeneity of a sample population is assumed if baseline respiratory and metabolic data are similar between participants. However, the present data suggests that muscle fibre type distribution in combination with the chosen exercise protocol may also play a role in whether ergogenic benefit is observed. In highly aerobic performance trials (i.e. ~ 85% aerobic ATP; Gastin et al. 1995) such as T_{LIM} at 100% of maximal power output (~ 4 to 8 minutes; MacLaren and Morgan 1985) individuals with a greater proportion of ST fibres, such as in SOL, may be more likely to see ergogenic benefit with $NaHCO_3$ than those individuals with a greater proportion of FT fibres. Similarly, those individuals with a greater proportion of FT fibres are more likely to see ergogenic benefit with $NaHCO_3$ in short performance trials of ~ 60 seconds where anaerobic metabolism (~ 50% anaerobic ATP; Gastin et al. 1995) is more predominant. Although analysing the fibre type distribution of human participants may be impractical, researchers should ensure homogeneity of volunteers (if that is required in the sample population) is not solely decided on similar respiratory and metabolic data in combination with a similar range of power output to body mass ratios. For example, researchers could consider incorporating detailed somatotyping into participant recruitment. As an individual's somatotype is a useful shorthand depiction of overall physique, in terms of body shape and composition independent of body size (Carter et al. 2005), including such preliminary analysis might reduce the variation in performance offering greater reliability of the collected data.

One of the limitations of the present study is that we have only evaluated the effects of $NaHCO_3$ on maximally (predominantly anaerobic type activity) stimulated skeletal muscle. Further work on the effects of $NaHCO_3$ on submaximally stimulated muscle, as has been considered in other ergogenic aids often used by humans to enhance physical performance (Tallis et al. 2012), is warranted.

In summary, isolated mouse EDL and SOL muscles incubated at 37°C in ~ 32 mM [HCO_3^-] generated greater maximal acute PO during cyclical contractions than CON muscles incubated in ~ 25 mM [HCO_3^-]. The elevated PO was due to greater force production throughout shortening. The ergogenic effect in EDL was double that of SOL. These results present the best indication to date that $NaHCO_3$ might have direct peripheral effects on mammalian skeletal muscle. Such results have potentially important implications for human exercise performance and training. Although significant differences were not observed in whole group data, the fatigability of muscle performance was variable suggesting, at the muscular level at least, that there may be inter-individual differences in response to $NaHCO_3$ supplementation. Such responses also differed between SOL and EDL and potentially will also differ between other muscles of differing muscle fibre composition. The overall results suggest that mammalian morphology and associated muscle fibre distribution might impact on the efficacy of $NaHCO_3$ as an ergogenic aid.

Chapter 7 – The effects of 6 weeks high-intensity training on the efficacy of sodium bicarbonate ($NaHCO_3$) as an ergogenic aid

7.1 Abstract

This study evaluated the effects of 6 weeks high-intensity cycling training on the efficacy of sodium bicarbonate ($NaHCO_3$) as an ergogenic aid. Ten healthy, non-cycling trained males (age 24.3 ± 5.8 years, height 179 ± 6 cm, pre-training body mass 81.0 ± 15.8 kg, W_{PEAK} 247 ± 30 W, $\dot{V}O_{2PEAK}$ 43 ± 9 $ml.kg^{-1}.min^{-1}$) performed a graded incremental exercise test, two familiarisation trials and two experimental trials before undertaking 6 weeks high-intensity cycling training. Experimental trials, which were repeated post-training after a further incremental test were counterbalanced and consisted of cycling to volitional exhaustion at 100% W_{PEAK} (T_{LIM}) 60 mins after ingesting either 0.3 $g.kg^{-1}$ body mass sodium bicarbonate ($NaHCO_3$) or 0.1 $g.kg^{-1}$ body mass sodium chloride (NaCl; PLA). Training consisted of three sessions per week including; (1) repeated short sprints (6, 8 and 10 s), (2) repeated longer duration sprints (30 s), both with a load equivalent to 7.5% body mass and (3) one bout of T_{LIM} at 100% W_{PEAK}. Training was completed 3 times per week with at least one rest day between sessions. Due to severe gastro-intestinal discomfort in 2 participants and to avoid clear bias of results they were removed from the main analysis. At the group level, pre-training T_{LIM} was 10% greater with $NaHCO_3$ than PLA (P = 0.06, ES = 0.4, benefit to harm odds ratio (OR) = 571). Post-training W_{PEAK} increased by 12 ± 7% (279 ± 30 W) although subsequent group level T_{LIM} was no greater than daily variation for $NaHCO_3$ compared to PLA (6%; P = 0.38, ES = 0.3, OR = 17). Some individual variation was observed for pre and post-training T_{LIM} performance between treatments although this was less marked than in studies 2 and 3. At the group level, based on the recommended benefit to harm odds-ratio of > 66, $NaHCO_3$ would be recommended for T_{LIM} at 100% W_{PEAK} before but not after 6 weeks high-intensity training. However, due to individual variation an individualised approach should be considered in an applied setting. In summary, at a group

level 6 weeks high-intensity cycling training reduces the effectiveness of $NaHCO_3$ in previously non-cycling trained males. Although the exact mechanisms remain to be elucidated, such changes in efficacy are likely due to, at least in part, training induced changes in intracellular buffering capacity.

7.2 Introduction

Substantial ionic and metabolic changes are observed in skeletal muscle following several weeks of repeated high-intensity sprint training (MacDougall et al. 1998, Burgomaster et al. 2005, Burgomaster, Heigenhauser, and Gibala 2006, Edge et al. 2006, Burgomaster et al. 2008). Suzuki et al. (2004) reported that mean power output (MPO) and peak power output (PPO) increased by ~ 9% and ~ 7%, respectively, after training consisting of single or double bouts of WAnT sprints, twice a week for 8 weeks. Similarly, MacDougall et al. (1998) reported significant increases in Wingate (WAnT) PPO (~ 23%) and TWD (~ 5%) after 7 weeks of repeated sprint training. The training, which consisted of increasing repeated WAnT (4 to 10 reps) interspersed with decreasing short recovery periods (4 to 2.5 mins), was performed three times per week and also improved $\dot{V}O_{2MAX}$ by ~ 7%. MacDougall et al. (1998) also reported significant increases in both glycolytic and oxidative enzymes leading the authors to suggest that increased PPO might have been due to increased maximal glycolytic enzyme activity and Na^+-K^+ pump activity. Interestingly, as little as 6 sessions over 2 weeks of high-intensity interval training can also induce significant metabolic benefits leading to improved exercise performance (Little et al. 2010).

Differences in participant training status across studies might help to explain why research evaluating the efficacy of extracellular buffers, such as sodium bicarbonate ($NaHCO_3$), demonstrate equivocal results (Requena et al. 2005, McNaughton, Siegler, and Midgley 2008, Peart, Siegler and Vince 2012). For example, Aschenbach et al. (2000) suggested that the highly trained wrestlers in their study might already possess a high

intracellular buffering capacity which left little opportunity for enhanced extracellular buffering to be effective. In addition to high levels of physical fitness, Linderman et al. (1992) suggested that their highly trained cyclists might have also been able to tolerate exercise discomfort better than untrained individuals contributing to why no ergogenic benefit was observed with $NaHCO_3$. Moreover, a recent meta-analysis demonstrated that the mean effect of $NaHCO_3$ on exercise performance in untrained individuals was 227% greater (ES 0.59 vs. 0.18) than in trained individuals (Peart, Siegler, and Vince 2012). However, it should be acknowledged that ergogenic benefit with $NaHCO_3$ supplementation has been reported in trained runners (Goldfinch, McNaughton, and Davies 1988, Bird, Wiles, and Robbins 1995) and cyclists (Driller et al. 2012a,b).

Although extracellular buffering systems (such as [HCO_3^-]) play a key role during exercise, the intracellular buffering capacity is crucial during high-intensity exercise (Parkhouse and McKenzie 1984, Parkhouse et al. 1985). Parkhouse et al. (1985) reported that highly trained runners (800 m) and rowers had significantly greater overall buffering capacity (+47%) and levels of carnosine (+53%), than highly trained marathon runners and untrained controls, respectively. It was speculated that such adaptations were the result of repetitive high-intensity exercise. This improved buffer capacity is supported by Suzuki et al. (2004) who reported a 113% increase in intramuscular carnosine concentration after 8 weeks cycling training consisting of single or double bouts of WAnT sprints, twice a week for 8 weeks. Therefore, it appears that intracellular buffers, such as carnosine, play an important role in the homeostasis of muscle cells during high-intensity exercise (Derave et al. 2010). Moreover, high-intensity training induced increases in carnosine have been implicated in performance improvement in untrained (Suzuki et al. 2004) and trained (Derave et al. 2007) participants.

Although a number of studies have examined the effects of $NaHCO_3$ ingestion *during* high-intensity training on a variety of physiological and performance parameters (Edge,

Bishop, and Goodman 2006, Thomas et al. 2007, Bishop et al. 2010), no studies have reported the efficacy of $NaHCO_3$ supplementation after an improvement in training status. Such an investigation is important as it would experimentally address issues raised from previous studies (Aschenbach 2000) and might reduce the equivocal nature of results in this body of research. Furthermore, by comparing the efficacy of $NaHCO_3$ before and after training in the same population we reduce the possible error from genetic differences in comparing populations from different studies. Moreover, it is important for training studies to address specific physiological adaptations which might explain training induced changes in physical performance (Noakes 2000). As an individual's training status might affect responses to $NaHCO_3$ during exercise (Linderman et al. 1992, Aschenbach et al. 2000) we evaluated the efficacy of $NaHCO_3$ on exercise capacity in non-cycling trained males before and after 6 weeks high-intensity cycling training. We hypothesised that 6 weeks high-intensity cycling training would alter the efficacy of $NaHCO_3$ in enhancing exercise capacity. More specifically, we hypothesise that pre-training, $NaHCO_3$ ingestion will enhance performance time (T_{LIM}) at 100% W_{PEAK} in untrained males. In contrast, after 6 weeks high-intensity cycling training we suggest that $NaHCO_3$ will not enhance performance time (T_{LIM}) at 100% W_{PEAK}.

7.3 Methods

7.3.1 Participants

Ten healthy, non-cycling trained males (age 24.3 ± 5.8 years, height 179 ± 6 cm, pre-training; body mass 81.0 ± 15.8 kg, $\dot{V}O_{2PEAK}$ 43 ± 9 $ml.kg^{-1}.min^{-1}$,W_{PEAK} 247 ± 30 W) volunteered for this study which had received University Ethics Committee approval. All participants were recreationally active undertaking 2 to 3 exercise sessions per week in a range of sports (e.g. football, rugby, swimming, badminton and/or running). None were specifically cycling trained.

7.3.2 Study design

Initially, participants visited the laboratory on 5 occasions prior to 6 weeks high-intensity cycling training. The first 5 sessions included an initial graded incremental exercise test (section 3.6.1) to determine $\dot{V}O_{2PEAK}$ and peak mean minute power (W_{PEAK}), two familiarisation and two experimental trials. The familiarisation and experimental trials consisted of cycling to volitional exhaustion at a constant load equivalent to 100% W_{PEAK} at 70 rev.min^{-1} (T_{LIM}; section 3.6.2). The training period consisted of 6 weeks high-intensity cycling (3 sessions per week) each interspersed by one full rest day (i.e. training on Monday, Wednesday, Friday; Burgomaster et al. 2008, Little et al. 2010). On completion of the training participants completed a second incremental test to determine post-training $\dot{V}O_{2PEAK}$ and W_{PEAK} and two experimental trials at post-training 100% W_{PEAK}. The post-training trials were identical to the pre-training trials as previously described. Participant screening and pre-experimental procedures are outlined in section 3.2. Figure 7.1 provides a visual schematic of the study design.

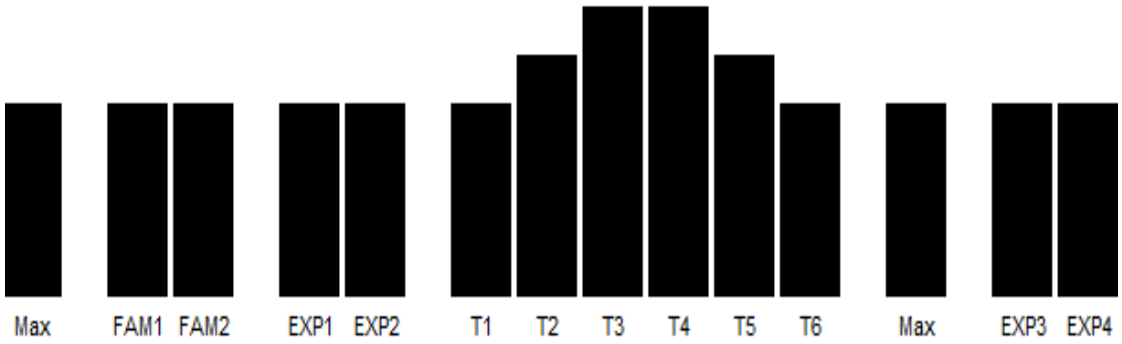

Figure 7.1 Overview of experimental design for study 4 (Max = graded incremental test, FAM = familiarisation trial, EXP = experimental trial, T(n) = training week (x 3 sessions per week). For T1 to T6, increasing/decreasing bars represent increase/decrease of training load.

7.3.3 Pre-training and post-training exercise protocols

On the first visit to the laboratory participants completed a graded incremental exercise test to determine $\dot{V}O_{2PEAK}$ and W_{PEAK} as described in section 3.6.1. Based on the results from study 1 (chapter 4) participants subsequently undertook two familiarisation trials at 100% W_{PEAK}. The bias between familiarisation trials (n=8) was 12 s / 4% which was lower than both the differences in T1 and T2 (24 s / 11%) and T2 and T3 (16 s / 6%) reported in study 1 (chapter 4). The lower bias observed in the present study is likely due to the highly motivated participants who were all very experienced with the T_{LIM} protocol. On the fourth and fifth visits, participants cycled to volitional exhaustion at a constant load equivalent to 100% W_{PEAK} (T_{LIM}) at 70 rev.min^{-1}, 60 mins after consuming either 0.3 g.kg^{-1} body mass $NaHCO_3$ or 0.1 g.kg^{-1} body mass NaCl (PLA) as described in section 3.6.2. Section 3.7 describes the treatment administration in more detail.

After five minutes seated resting heart rate (HR, section 3.3.4), perceived readiness to exercise (PRE; Nurmekivi et al. 2001, section 10.3), abdominal discomfort (AD) and gut fullness (GF; Price, Moss, and Rance 2003, section 3.4.2) were recorded. Blood samples were then taken for blood lactate concentration ([BLa]), pH, base excess (BE) and bicarbonate ion concentration ($[HCO_3^-]$). Blood was collected and analysed as outlined in sections 3.5.1 and 3.5.2. After baseline measurements were completed the participant consumed the $NaHCO_3$ or PLA drink within the first 5 mins of the 60 mins pre-exercise period (Price and Simons 2010). Participants remained seated throughout and were allowed to consume water *ad libitum* to minimise gastrointestinal (GI) discomfort. The mean volume of water consumed was monitored and estimated at ~ 350 ml. Perceived readiness to exercise (PRE), AD and GF were recorded at 30 mins and 60 mins following ingestion. At 60 mins following ingestion HR was recorded and further blood samples taken for BLa and pH.

Forty-five minutes after ingestion, participants started breathing into the breath-by-breath gas collection system as previously indicated (section 3.3.3). Baseline data was averaged over the last sixty seconds of the pre-exercise period and for the last ten seconds

of exercise. Expired gas was measured for $\dot{V}_E$ and calculated for $\dot{V}O_2$ and RER, respectively. Upon completion of baseline data collection, the participant completed the T_{LIM} test at 100% W_{PEAK} as described in section 3.6.2. Ratings of perceived exertion (RPE; 6-20 scale) (Borg, 1982) for local RPE (RPE_L), representing the exercising muscles, and overall RPE (RPE_O), reflective of cardiovascular strain were recorded as described in section 3.4.1. Abdominal discomfort, GF and HR were recorded and blood samples taken for BLa, pH, BE and [HCO_3^-] immediately post-exercise. Final blood samples were taken 5 minutes post-exercise. Upon completion of the test, the participant was encouraged to cycle for 5 minutes at 70 W to warm down and avoid syncope.

7.3.4 Six weeks high-intensity training protocol

The 6-week training programme was based on several principles of training, including overload, progression and specificity as described by Baechle and Earle (2008). Table 7.1 provides an overview of the training programme which was tapered in the final weeks to minimise possible effects of overtraining, as per previous research (Burgomaster et al. 2005, Edge, Bishop, and Goodman 2006). Participants completed eighteen supervised training sessions performed on a cycle ergometer (Monark 824E Ergomedic, Monark, Varberg, Sweden). Over the course of the training period participants were asked to continue with normal diet, activity and usual training commitments. Most sessions (>95%) were completed in pairs to encourage intra and inter session competition and attendance. Overall session adherence was 100%. Participants completed each training session separated by one or more full days (i.e. Monday, Wednesday and Friday). The first session was undertaken ~ 3 days after the final pre-training experimental trial. Prior to all sessions participants warmed up by cycling at 70 $rev.min^{-1}$ for 4 mins at 50% $W_{PEAK,}$ 1 min at 75% W_{PEAK} and then 2 mins at 70 W. This warm up was consistent with that used in the pre-training experimental T_{LIM} trials. The resistance set for 100% W_{PEAK} was calculated from the initial incremental test (section 3.6.1).

Table 7.1 Summary of 6 week high-intensity cycling training programme

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Session 1 (Overload / Progression)						
No. sets	2	2	2	2	2	2
No. sprints per set	10	10	10	10	10	10
Duration (s)	6	8	10	10	8	6
Between bout recovery (s)	30	30	30	30	30	30
Between set recovery (s)	300	300	300	300	300	300
Session 2 (Specificity / Overload / Progression)						
No. sets	1	1	1	1	1	1
No. bouts per set	1	1	1	1	1	1
Duration (s)	T_{LIM}	T_{LIM}	T_{LIM}	T_{LIM}	T_{LIM}	T_{LIM}
Session 3 (Overload / Progression)						
No. sets	1	1	1	1	1	1
No. bouts per set	3	4	5	5	4	3
Duration (s)	30	30	30	30	30	30
Between bout recovery (s)	300	300	300	300	300	300

Note: T_{LIM} = time to volitional exhaustion at 100% W_{PEAK}

Session 1 (aims: overload, progression, taper) involved two bouts of ten short sprints against a load of 7.5% body mass which was established prior to week 1 and week 4 (i.e. mid way through training). Recovery between sprints was kept constant at 30 s and between bouts at 300 s. Sprint duration increased from 6 s to 8 s to 10 s (weeks 1, 2 and 3 respectively) and decreased from 10 s to 8 s to 6 s (weeks 4, 5 and 6 respectively). Session 3 (aims: overload, progression, taper) involved 30 s sprints against a load of 7.5% body mass, calculated as noted above. The number of sprints increased from 3 to 4 to 5 (weeks 1, 2 and 3, respectively) and decreased from 5 to 4 to 3 (weeks 4, 5 and 6 respectively). At the end of each third session (i.e. every Friday) participants were told their peak power output (PPO) and mean power output (MPO) for each sprint. For both sessions 1 and 3, a taper was incorporated to minimise possible negative effects of overtraining.

Session 2 (aims: overload, progression and specificity) involved completing one T_{LIM} bout at pre-training 100% W_{PEAK} as per experimental trials. In contrast to experimental trials, for T_{LIM} sessions during training participants were allowed to see the clock and were told their times from week 2 onwards to enhance motivation and intra and inter participant competition. Indeed, anecdotal feedback from participants suggested that the competitive element during training was very beneficial both from a psychological (goal setting, alleviating boredom, achievement, adherence) as well as physiological (increased time/power for similar perceived effort, quicker recovery between bouts/sets) perspectives. On successful completion of the training programme participants performed the post-training incremental test and final two experimental trials after 2 to 3 full days rest. Ratings of perceived exertion (RPE 6-20, Borg, 1982) for RPE_L and RPE_O (Robertson et al. 1986, Swank and Robertson 1989) were recorded at the end of every training session to provide an indication of training intensity.

A stationary start was chosen for sessions 1 and 3 as it facilitates higher peak power in anaerobic sprint tests compared to rolling starts (Coleman, Hale, and Hamley 1985) and affords greater consistency with participants starting with the same pedal position (Lavender and Bird 1989). A stationary start was also employed for training session 2 which has previously been used in evaluating high-intensity cycling in a laboratory setting with active but not specifically cycling trained males, similar to the present study (Wittekind, Micklewright and Beneke 2011). This starting procedure is consistent with the familiarisation and performance tests in studies 1 (chapter 4) and 2 (chapter 5), respectively.

7.3.5 Statistical analysis

Statistical analysis was completed using SPSS (IBM v17 and 20, Chicago, USA). Statistical significance, normality and homogeneity of variance/sphericity of data was assessed / adjusted as outlined in section 3.8. All cardiorespiratory, perceptual and blood

variables were analysed by 3-way (training status * treatment * time) repeated measures ANOVA. Where significance was achieved for main effects pairwise comparisons (least significant difference; LSD) were undertaken. LSD comparisons were chosen as they are the most powerful when analysing 3 levels/groups (Maxwell and Delaney 2004, Cardinal and Aitken 2006, Howell 2007). For interactions, Tukeys' post hoc analysis was undertaken by calculating the difference required between means for significance at the level of $P < 0.05$ (Vincent 1999). The time points considered for HR and blood variables were pre-ingestion (-60), pre-exercise (0), immediately post-exercise and five minutes post-exercise. Respiratory data ($\dot{V}O_2$, $\dot{V}_E$ and RER) was considered at rest and during the final 10 s of exercise. Values for RPE_L and RPE_O were analysed at 1 min, 2 mins, and 3 mins during exercise and at volitional exhaustion. AD and GF were analysed pre-ingestion, 30 mins post-ingestion, pre-exercise and post-exercise. Finally, PRE was analysed pre-ingestion, 30 mins post-ingestion and pre-exercise.

Correlation coefficients (Spearman's ρ and Pearson's r for non-parametric and parametric data, respectively) and effect sizes (ES) are reported where appropriate. For ANOVA, ES are reported as the partial η^2 value and for between trial comparisons ES was calculated using the difference in means divided by the pooled SD of the compared trials. Magnitude based inferences are presented where appropriate (section 3.8.1; Hopkins et al. 2009). Odds ratios are also presented for T_{LIM} data where > 66 represents the recommended benefit: harm threshold (section 3.8.1).

7.4 Results

Two of the ten participants suffered severe gastrointestinal (GI) distress (6 or 7 on 11-point Likert scale) during one of their $NaHCO_3$ trials (one pre-training, one post-training) which contributed to large decreases in T_{LIM} performance when compared to PLA (-29% and -33%, respectively). Table 7.2 demonstrates that the relationship between differences in T_{LIM}

between treatments and quantity of $NaHCO_3$ consumed changes substantially between n=8 and n=10 despite no difference in $NaHCO_3$ absolute load in any sub-group (P = 0.82). Figure 7.2 demonstrates that these performance outliers were outside of 95% limits of agreement. Therefore, these participants were removed from the main analysis to avoid presenting biased results (Saunders et al. 2011).

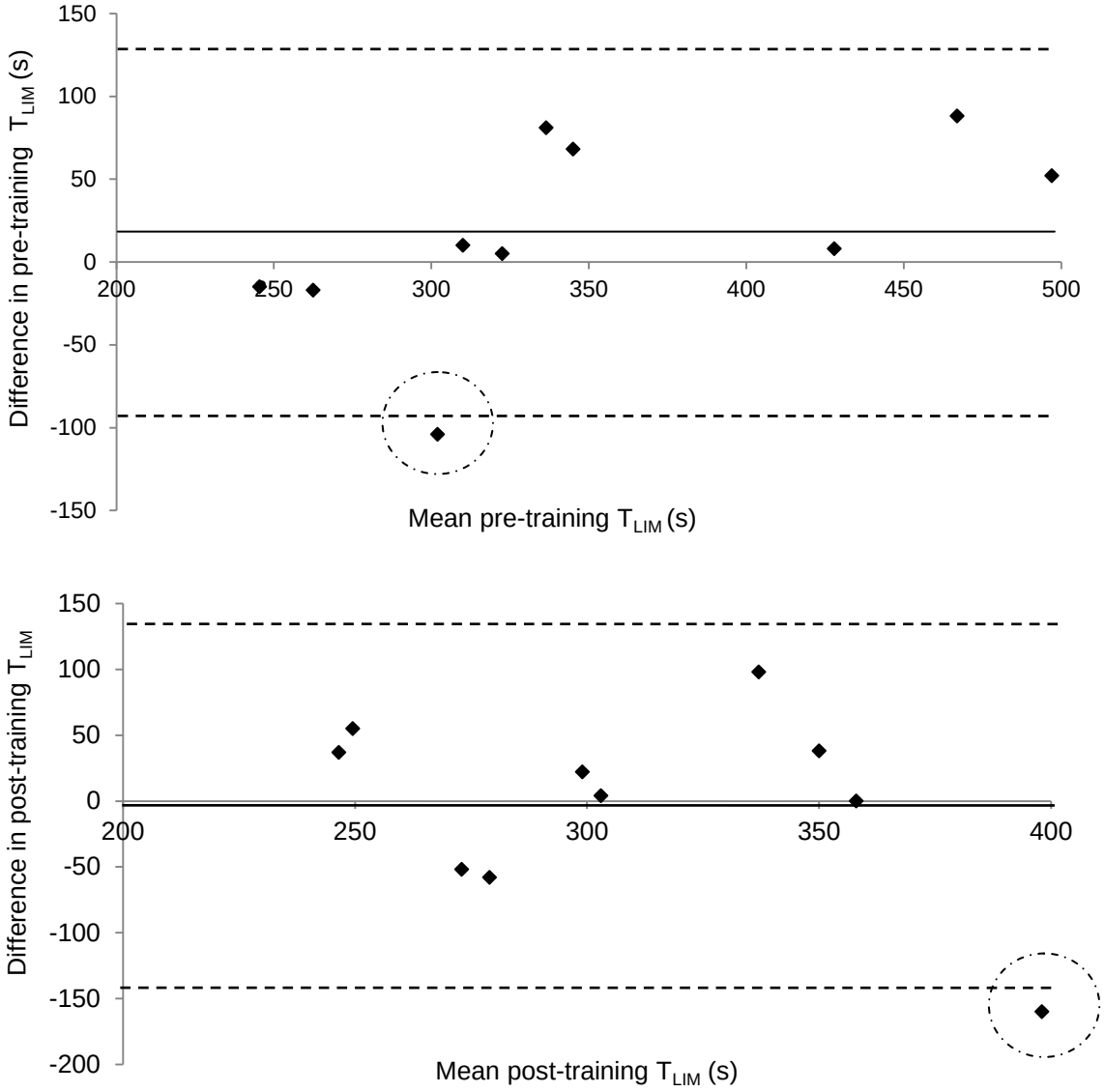

Figure 7.2 Bland-Altman plots for pre-training (top) and post-training (bottom) 100% W_{PEAK} T_{LIM} between $NaHCO_3$ and PLA (n=10). Dotted lines represent limits of agreement (± 1.96 SDs; 95% confidence) and solid line is mean bias between trials. Dotted circles represent significant performance outliers.

It should be noted that other participants did suffer similar GI distress or had lowered T_{LIM} with $NaHCO_3$ compared to PLA. However, such results were not as extreme reactions or outliers and were therefore included in the analysis. After removing the two participants from the analysis W_{PEAK} and $\dot{V}O_{2PEAK}$ values recorded during the initial incremental test remained similar for the group (n=8; 249 ± 34 W and 46 ± 8 $ml.kg^{-1}.min^{-1}$, n=10; 247 ± 30 W and 43 ± 9 $ml.kg^{-1}.min^{-1}$). There was no significant difference (P = 0.28) in 100% W_{PEAK} between the present study (249 ± 34 W) and study 2 (228 ± 37 W).

Table 7.2 Correlation data for differences in T_{LIM} between treatments and absolute quantity of $NaHCO_3$ consumed (n=8 and n=10) pre and post-training.

n = 8	**Mean Diff T_{LIM} (s)**	**r**	**P**	**$NaHCO_3$ (g)**
Pre-training	34	0.70	0.055	22.8 ± 3.8
Post-training	18	0.70	0.052	23.1 ± 3.7
n = 10	**Mean Diff T_{LIM} (s)**	**r**	**P**	**$NaHCO_3$ (g)**
Pre-training	17	-0.19	0.60	24.3 ± 4.7
Post-training	-1	0.08	0.83	24.6 ± 4.6

Table 7.3 Summary of pre and post-training incremental test data (n=8)

	Pre	**Post**	**% change (± SE)**	**P**	**ES**
Body Mass (kg)	76.0 ± 12.6	77.2 ± 12.2	1.5 ± 1.8	0.15	0.1
BMI	23.7 ± 3.0	24.0 ± 2.8	1.5 ± 1.8	0.20	0.1
W_{PEAK} (Watts)	249 ± 34	279 ± 30	12 ± 7	0.009 *	0.9
$\dot{V}O_2$ ($l.min^{-1}$)	3.43 ± 0.42	4.00 ± 0.55	17 ± 10	0.02 *	1.2
$\dot{V}O_2$ ($ml.kg^{-1}.min^{-1}$)	46 ± 9	52 ± 7	14 ± 11	0.02 *	0.8
$\dot{V}_E$ ($l.min_{-1}$)	137 ± 22	144 ± 23	5 ± 16	0.45	0.3
RER	1.12 ± 0.07	1.08 ± 0.09	-4 ± 8	0.37	0.5
Pre-exercise HR (bpm^{-1})	71 ± 8	61 ± 13	-14 ± 9	0.02 *	0.9
Final HR (bpm^{-1})	188 ± 6	186 ± 10 #	-1 ± 2	0.62	0.2
Post-ex BLa ($mmol.l^{-1}$)	11.3 ± 1.5	12.1 ± 2.9	7 ± 17	0.48	0.3
5 mins Post BLa ($mmol.l^{-1}$)	10.6 ± 1.5	10.6 ± 2.8	0.4 ± 18	0.97	0.02
RPE_O	20.0 ± 0.0	19.3 ± 1.2	-4 ± 4	0.11	0.9

Note: # n=7,* P < 0.05

7.4.1 Preliminary tests

Table 7.3 outlines the physiological results from the incremental test for $\dot{V}O_{2PEAK}$ and W_{PEAK} before and after training (n=8). After 6 weeks high-intensity training W_{PEAK} increased by 12 ± 7 % (ES = 0.9). In support of this change, both absolute $\dot{V}O_2$ ($l.min^{-1}$) and relative $\dot{V}O_2$ ($ml.kg^{-1}.min^{-1}$) improved by 17 ± 10 % (ES = 1.2) and 14 ± 11 % (ES = 0.8) respectively. Interestingly, there was a 14 ± 9 % reduction in resting HR (Table 7.3). The data outlined in table 7.3 demonstrate that participants reached the criteria for a valid peak oxygen uptake test as outlined by Bird and Davison (1997).

7.4.2 Efficacy of $NaHCO_3$ ingestion pre and post 6 weeks high-intensity cycle training

Tables 7.4 and 7.5 show performance data and benefit to harm odds ratios of PLA and $NaHCO_3$ treatments for both n=8 and n=10 pre and post 6 weeks high intensity training.

Table 7.4 Performance time (T_{LIM}) for n=8 and n=10 pre and post 6 weeks training

Training	PLA T_{LIM} (s)	$NaHCO_3$ T_{LIM} (s)	T_{LIM} Diff (s)	% Change	P	ES
Pre (n=8)	331 ± 76	365 ± 106	34 ± 31	10 ± 9%	0.06	0.37
Post (n=8)	290 ± 47	308 ± 56	18 ± 37	6 ± 13%	0.38	0.34
Pre (n=10)	343 ± 73	360 ± 104	17 ± 41	5% ± 12%	0.36	0.20
Post (n=10)	310 ± 72	309 ± 50	-1 ± 51	-1% ± 15%	0.95	0.03

Note: T_{LIM} = ± SD, T_{LIM} Diff / % Change = ± SE

Table 7.5 Probability of beneficial, trivial or harmful outcomes (T_{LIM}) for n=8 and n=10 pre and post 6 weeks high-intensity training based on smallest worthwhile change (SWC)

Training	SWC	Beneficial	Trivial	Harmful	Benefit: Harm Odds Ratio
Pre (n=8)	2.7%	32.6%	67.3%	0.1%	571
Post (n=8)	2.2%	12.4%	86.8%	0.8%	17
Pre (n=10)	2.5%	7.9%	91.7%	0.4%	24
Post (n=10)	2.0%	3.5%	92.2%	4.4%	0.8

Note: SWC based on pre and post incremental test for W_{PEAK}

Pre-training (n=8), T_{LIM} was 10% greater with $NaHCO_3$ than PLA which equates to a 32.6% chance of beneficial change and a benefit to harm odds-ratio of 571. Post-training (n=8) T_{LIM} was 6% greater with $NaHCO_3$ than PLA although this was not significant. In contrast to pre-training this equated to a 12.4% chance of beneficial change and a benefit to harm odds-ratio of 17. Pre-training (n=10), T_{LIM} was 5% greater with $NaHCO_3$ than PLA although this was not significant. This equated to an 8% chance of beneficial change and a benefit to harm odds-ratio of 24. Post-training (n=10) T_{LIM} was 1% lower with $NaHCO_3$ than PLA which equated to a benefit to harm odds-ratio of 0.8 (Tables 7.4, 7.5). For n=8, traditional statistical analysis suggests that there was no order effect pre-training (P = 0.48) or post-training (P = 0.18) for T_{LIM} at 100% W_{PEAK}. However, post-training the final T_{LIM} trial was 8% lower (ES = 0.5) than the penultimate T_{LIM} trial regardless of treatment (ES = 0.1 for pre-training). The reason is unclear but might be due to a reduction in motivation and/or increase in fatigue perception based on the knowledge that it was the last trial after an intensive training program.

7.4.3 Abdominal discomfort (AD) and gut fullness (GF)

Although there were no interactions for AD, there was a main effect for treatment (P = 0.019, ES = 0.6) demonstrating that AD was greater for $NaHCO_3$ than PLA by 1.2 units (1.9 ± 2.3 vs. 0.7 ± 0.9). However, overall AD was low (1.3 ± 1.8). There were no main effects or interactions for GF (P > 0.05) with treatments exhibiting almost identical GF scores ($NaHCO_3$: 2.2 ± 2.0 and PLA: 2.0 ± 1.9). The main effect for time and interaction for treatment * time approached significance (P = 0.087 and P = 0.081, respectively). Similarly to AD, overall GF was low (2.1 ± 1.9).

7.4.4 Ratings of perceived exertion (RPE)

7.4.4.i Local (RPE_L)

There was a treatment * training status * time interaction for RPE_L (P = 0.05; ES = 0.3). After 2 mins exercise pre-training RPE_L was lower for $NaHCO_3$ vs. PLA (14.4 ± 1.4 vs. 15.3 ± 1.5, respectively; P < 0.01) but similar post-training (16.0 ± 1.1 vs. 16.1 ± 1.6, respectively, Figure 7.3). Despite similar RPE_L ratings after 1 min and at the end of exercise, RPE_L after 2 mins and 3 mins was lower pre compared to post-training (14.8 ± 1.5 vs. 16.1 ± 1.3 and 16.4 ± 1.4 vs. 17.4 ± 1.1 for 2 mins and 3 mins, respectively; P < 0.01).

7.4.4.ii Overall (RPE_O)

There were significant main effects for training status (P = 0.001, ES = 0.8) and time (P < 0.001, ES = 1.0) with the training status * time interaction approaching significance (P = 0.065, ES = 0.3). Overall, RPE_O was lower pre-training (13.4 ± 3.4) compared to post-training (14.7 ± 3.4). As expected, RPE_O increased over time with mean values of 11.1 ± 0.6, 13.3 ± 1.0, 14.7 ± 1.0 and 17.3 ± 0.4 after 1, 2 and 3 min and at the end of exercise, respectively.

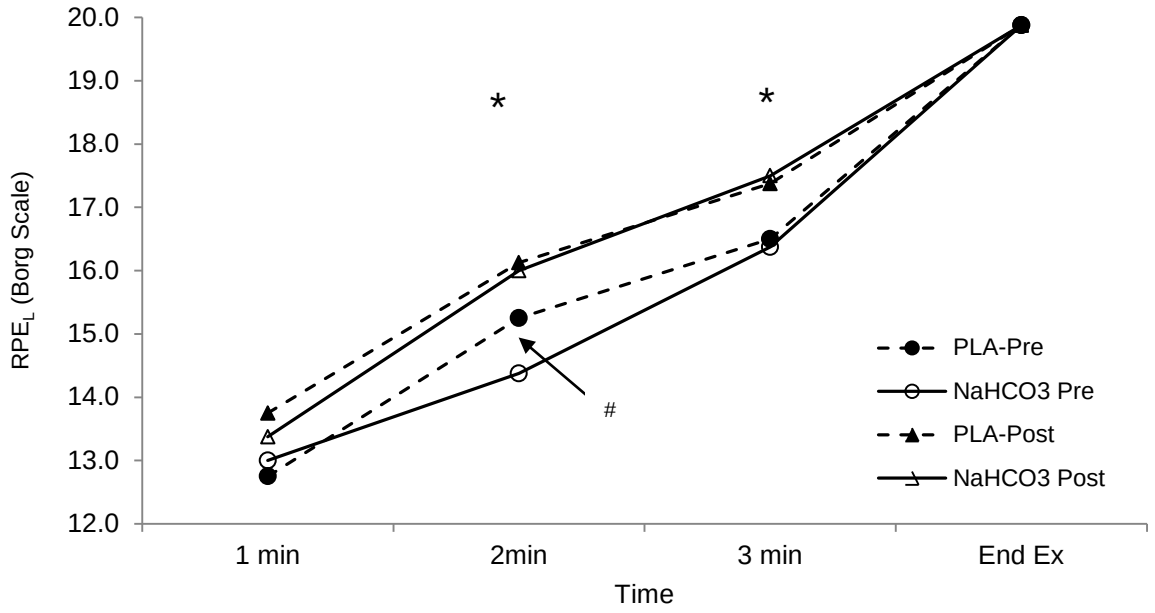

Figure 7.3 Localised rating of perceived exertion (RPE_L) after 1, 2,and 3 mins and at the end of T_{LIM} with $NaHCO_3$ and PLA, pre and post-training (Error bars omitted for clarity). $^{\#}$ $NaHCO_3$ < PLA pre-training (P < 0.01), * Pre-training < Post-training (P < 0.01).

7.4.5 Perceived readiness to exercise (PRE)

There were no main effects or interactions for PRE. Similar values for PRE were recorded 30 mins post ingestion (Pre-training: 8 ± 2 vs. 6 ± 2; Post-training: 7 ± 2 vs. 6 ± 3) and pre-exercise (Pre-training: 8 ± 2 vs. 6 ± 2; Post-training: 7 ± 2 vs. 6 ± 3) for PLA compared to $NaHCO_3$, respectively, both pre and post-training.

7.4.6 Heart rate (HR)

There were main effects for treatment ($P = 0.017$, ES = 0.6) and time ($P < 0.001$, ES = 1.0) for HR. Overall, ingestion of $NaHCO_3$ resulted in a higher mean HR than PLA (110 ± 48 vs. 105 ± 47 bpm^{-1}). The training status * Time ($P = 0.058$, ES = 0.3) and treatment * time ($P = 0.058$, ES = 0.3) interactions approached significance. As these interactions were close to significance, post-hoc Tukey calculations were carried out for illustrative purposes. At the end of exercise HR was higher pre-training (184 ± 8 bpm^{-1}) than post-training (177 ± 9 bpm^{-1}; $P < 0.05$) and HR was higher pre-exercise (71 ± 10 vs. 64 ± 9 bpm^{-1}) and 5 mins post-exercise (115 ± 10 vs. 108 ± 9 bpm^{-1}) for $NaHCO_3$ compared to PLA, respectively ($P < 0.05$).

7.4.7 Respiratory data

There was a main effect for time for $\dot{V}O_2$ ($P < 0.001$, ES = 1.0) but there were no further main effects or interactions. Oxygen consumption at rest (0.42 ± 0.04, 0.44 ± 0.08, 0.47 ± 0.08 and 0.47 ± 0.11 $l.min^{-1}$) and at the end of exercise (4.15 ± 0.37, 4.20 ± 0.57, 4.09 ± 0.90 and 4.15 ± 0.50 $l.min^{-1}$) were similar for pre-training PLA and $NaHCO_3$ and post-training PLA and $NaHCO_3$ trials, respectively. There was a main effect for time for $\dot{V}_E$ ($P < 0.001$, ES = 1.0) and the training status * time interaction approached significance ($P = 0.085$, ES = 0.4). A separate t-test revealed that $\dot{V}_E$ at exhaustion was lower post-training compared to pre-training (137.2 ± 21.2 vs. 149.0 ± 13.8 $l.min^{-1}$; $P = 0.02$, ES = 0.7).

Additionally, there was a treatment * time interaction for RER (P = 0.003, ES = 0.8). In general RER was greater in the post-training $NaHCO_3$ trial (1.14 ± 0.12) than both pre and post-training PLA trials (1.08 ± 0.10, 1.10 ± 0.13; P < 0.05) but not the pre-training $NaHCO_3$ trial (1.11 ± 0.10; Figure 7.4).

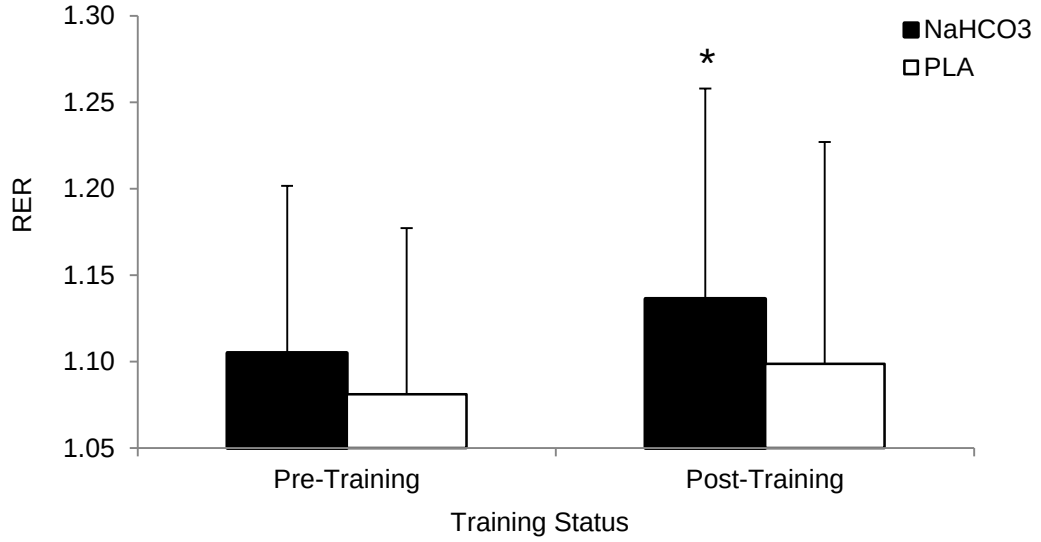

Figure 7.4 Post-exercise (T_{LIM}) respiratory exchange ratio (RER) for $NaHCO_3$ and PLA treatments, pre and post-training. * P < 0.05 compared to all PLA trials.

7.4.8 Blood data

7.4.8.i Blood Lactate (BLa) concentration

There were main effects for training status (P = 0.005, ES = 0.7), treatment (P = 0.001, ES = 0.8) and time (P < 0.001, ES = 1.0) for BLa. Additionally, there were training status * time (P = 0.01, ES = 0.6) and treatment * time (P < 0.001, ES = 0.8) interactions for BLa. Although there was no difference in BLa pre-ingestion (1.0 ± 0.5 vs. 0.8 ± 0.2 $mmol.l^{-1}$) or pre-exercise (1.0 ± 0.5 vs. 0.8 ± 0.3 $mmol.l^{-1}$), BLa was greater (P < 0.05) at the end of exercise (14.7 ± 3.3 vs. 12.1 ± 2.7 $mmol.l^{-1}$) and 5 mins post-exercise (13.0 ± 3.0 vs. 10.7 ± 2.8 $mmol.l^{-1}$; Figure 7.4) pre compared to post-training, respectively. Similarly, although comparable at rest and pre-exercise BLa was greater (P < 0.01) for $NaHCO_3$ compared to PLA at the end of exercise (15.2 ± 3.3 vs. 11.6 ± 2.0 $mmol.l^{-1}$) and 5 mins post-exercise

(13.3 ± 3.2 vs. 10.5 ± 2.2 mmol.l^{-1}; Figure 7.5). Interestingly, pre-training there was a significant correlation between the difference in BLa and the % difference in T_{LIM} between treatments at the end of exercise (r = 0.84, r^2 = 0.70, P = 0.01). The correlation between the difference in BLa and the % difference in T_{LIM} between treatments 5 mins post-exercise approached significance (r = 0.67, r^2 = 0.45, P = 0.07). No significant correlations for differences in BLa and % difference in T_{LIM} between treatments were observed post-training (both P > 0.3).

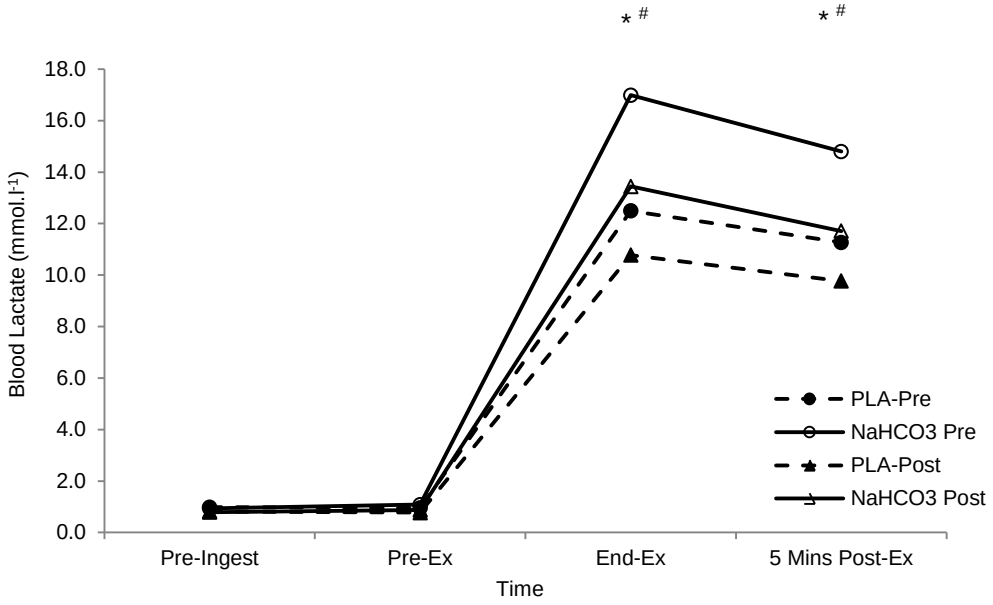

Figure 7.5 Blood lactate at pre-ingestion, pre-exercise, at the end of exercise and 5 mins post-exercise after $NaHCO_3$ or PLA, pre and post 6 weeks high intensity training. * Pre-training > post-training (P < 0.05), $^{\#}$ $NaHCO_3$ > PLA (P < 0.01).

7.4.8.ii pH

There were significant interactions for treatment * time (P < 0.001, ES = 0.7) and training status * time (P = 0.037, ES = 0.3) for pH. Despite being similar pre-ingestion (7.44 ± 0.02 vs. 7.42 ± 0.02) pH was 0.07 ± 0.02 units greater for $NaHCO_3$ compared to PLA pre-exercise (7.48 ± 0.02 vs. 7.41 ± 0.02), 0.09 ± 0.02 units greater post-exercise (7.31 ± 0.04 vs. 7.22 ± 0.05) and 0.08 ± 0.03 units greater 5 mins post-exercise (7.33 ± 0.04 vs. 7.25 ±

0.06; P < 0.01, Figure 7.6). Despite being similar pre-ingestion, pre-exercise and post-exercise, pH at 5 mins post-exercise was greater post-training (7.30 ± 0.07) compared to pre-training (7.28 ± 0.06; P < 0.05).

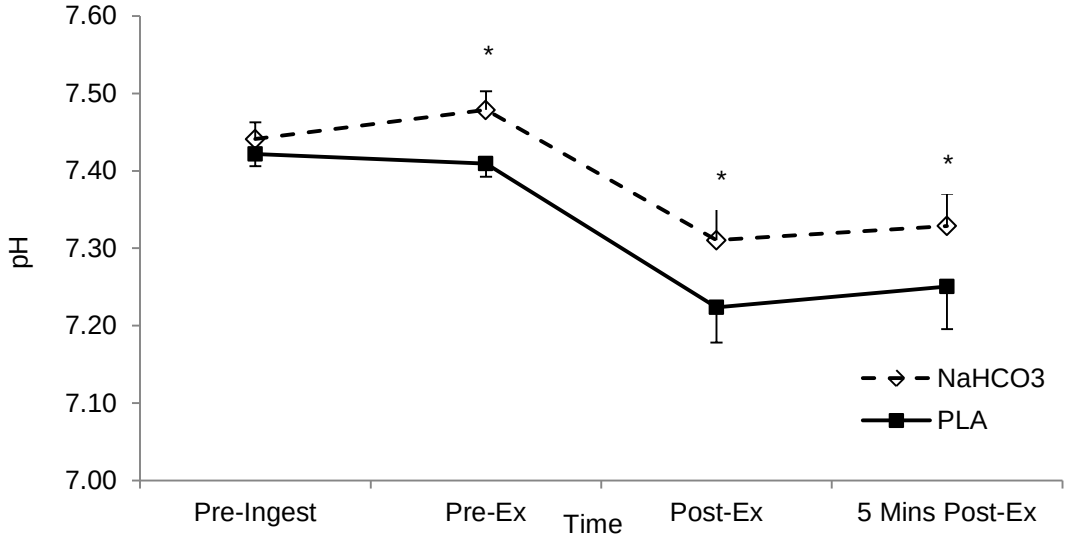

Figure 7.6 Blood pH over time for $NaHCO_3$ and PLA treatments. * $NaHCO_3$ > PLA (P < 0.01).

7.4.8.iii Base excess (BE)

There were significant treatment * time (P < 0.001, ES = 0.8, Table 7.6) and training status * time (P = 0.005, ES = 0.5) interactions for BE. Despite being similar pre-ingestion, pre-exercise and at the end of exercise, BE at 5 mins post-exercise was greater (P < 0.05) post-training (-9.4 ± 4.4 mmol.l^{-1}) compared to pre-training (-11.5 ± 3.5 mmol.l^{-1}).

Table 7.6 Summary of treatment * time interaction for BE and [HCO_3^-]

Variable	Treatment	Pre-Ingest	Pre-Ex	End-Ex	5 mins post Ex
BE (mmol.l^{-1})	$NaHCO_3$	0.8 ± 1.9	7.6 ± 1.3 *	-8.6 ± 2.7 *	-7.9 ± 3.1 *
	PLA	0.4 ± 1.5	-0.4 ± 1.7	-14.5 ± 2.8	-13.0 ± 3.2
[HCO_3^-](mmol.l^{-1})	$NaHCO_3$	24.0 ± 2.2	31.8 ± 1.6 *	16.3 ± 2.2 *	16.7 ± 2.7 *
	PLA	24.0 ± 1.7	23.5 ± 1.9	11.9 ± 2.3	12.6 ± 2.4

Note: * P < 0.01 for $NaHCO_3$ compared to PLA

7.4.8.iv Bicarbonate Ion Concentration ($[HCO_3^-]$)

There were significant treatment * time ($P < 0.001$, ES = 0.9) and training status * time ($P = 0.003$, ES = 0.5) interactions for $[HCO_3^-]$. Blood $[HCO_3^-]$ was greater ($P < 0.01$) for $NaHCO_3$ compared to PLA pre-exercise, at the end of exercise and 5 mins post-exercise (Table 7.6). Additionally, blood $[HCO_3^-]$ 5 mins post-exercise was greater ($P < 0.05$) post-training (15.7 ± 3.3 mmol.l^{-1}) compared to pre-training (13.6 ± 2.9 mmol.l^{-1}).

7.5 Training responses

7.5.1 Ratings of perceived exertion (RPE)

Post-exercise RPE_L and RPE_O followed a similar pattern throughout the 6-week high-intensity cycling training program for session 1 (Figure 7.7). Mean scores of 18.7 ± 1.8 and 16.7 ± 2.6 were noted for RPE_L and RPE_O, respectively, over the 6 week period. Between weeks 2 to 5 (i.e. hardest training), RPE_L was 19.4 ± 1.0. Between weeks 2 to 5, RPE_O was 17.5 ± 2. RPE_L was lowest at week 6 (17.0 ± 2.2) which was significantly lower than weeks 2-5 inclusive (all $P < 0.02$). RPE_O was also lowest at week 6 (14.0 ± 1.3) which was significantly lower than weeks 2, 3, 4 ($P < 0.01$) and 5 ($P < 0.02$) suggesting the tapering process, at least from a perceptual perspective, had the desired effect. Differences between week 1 and week 6 approached significance ($P = 0.056$; Figure 7.7). Post-exercise RPE_L after session 2 was not different during training (all comparisons $P > 0.05$). Mean post-exercise RPE_L was 19.9 ± 0.3. Post-exercise RPE_O was consistently lower than RPE_L with a mean score of 17.2 ± 2.6 over the 6 weeks training period. RPE_O peaked at week 5 (18.1 ± 2.6) which was significantly higher ($P = 0.03$) than week 1 (16.1 ± 3.4). No further differences in RPE_O were recorded (Figure 7.8). There were no differences in post-exercise RPE_L (19.8 ± 0.6) or RPE_O (16.8 ± 2.5) throughout the 6 weeks training period for session 3(all comparisons $P > 0.05$; Figure 7.9).

7.5.2 Exercise capacity (T_{LIM})

Table 7.7 shows each participant's T_{LIM} (session 2) for each week of the 6 weeks training program. Although there were no differences in T_{LIM} between weeks 4, 5 and 6, T_{LIM} was lower in weeks 1 (P = 0.036, P = 0.017 and P = 0.038) and 2 (P = 0.038, P = 0.031 and P = 0.042) when compared to weeks 4, 5 and 6, respectively. Additionally, T_{LIM} for week 3 was lower than weeks 5 (P = 0.024) and 6 (P = 0.028), respectively. T_{LIM} improved in a linear fashion with a mean improvement from week 1 of 149%. Although there was linear improvement over the 6 weeks, 5 out of 8 participants (63%) recorded their highest T_{LIM} in week 5, the remainder in week 6 (Table 7.7, Figure 7.10).

Table 7.7 Participant's T_{LIM} (s) during 6 weeks high-intensity training (**Note:** % = highest T_{LIM} in week 5 or 6 as a % improvement in relation to T_{LIM} week 1; **Bold** represents highest T_{LIM}).

	Training Week						
Participant	**1**	**2**	**3**	**4**	**5**	**6**	**%**
1	314	359	392	438	406	**500**	59%
2	487	631	791	981	1097	**1345**	176%
3	383	340	370	434	**629**	541	164%
4	342	310	268	289	**376**	323	110%
5	310	422	603	484	**899**	692	290%
6	380	562	606	683	485	**784**	106%
7	365	374	382	428	**518**	423	142%
8	328	368	269	421	**482**	371	147%
Mean	364	421	460	520	612	622	149%
SD	57	114	187	216	256	331	48%

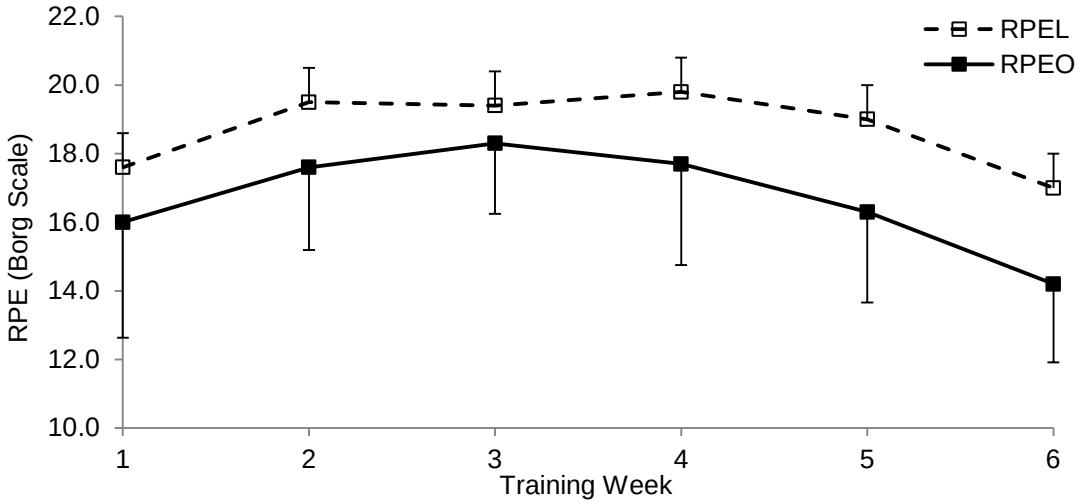

Figure 7.7 Post-exercise RPE_L and RPE_O for session 1 throughout training

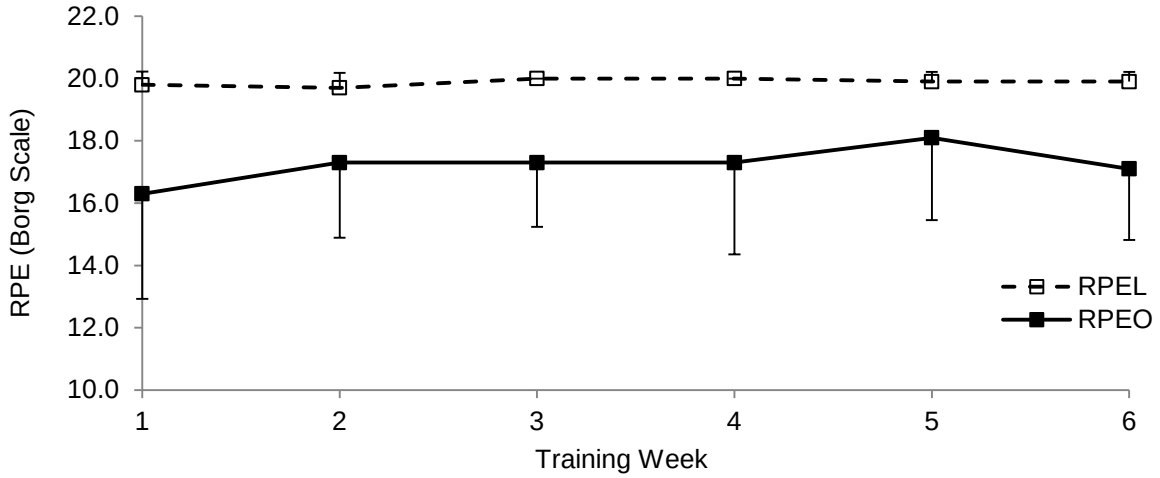

Figure 7.8 Post-exercise RPE_L and RPE_O for session 2 throughout training

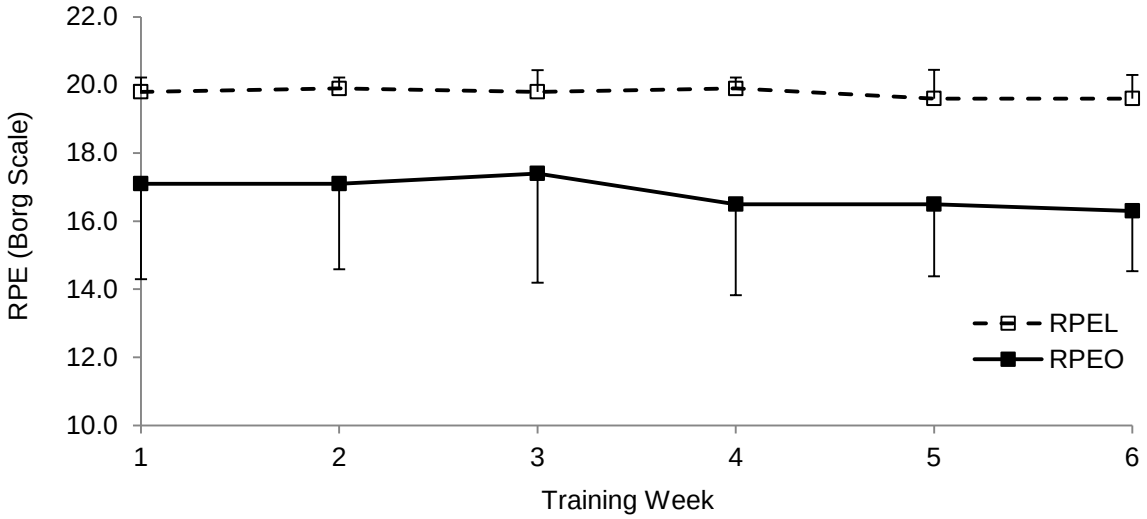

Figure 7.9 Post-exercise RPE_L and RPE_O for session 3 throughout training

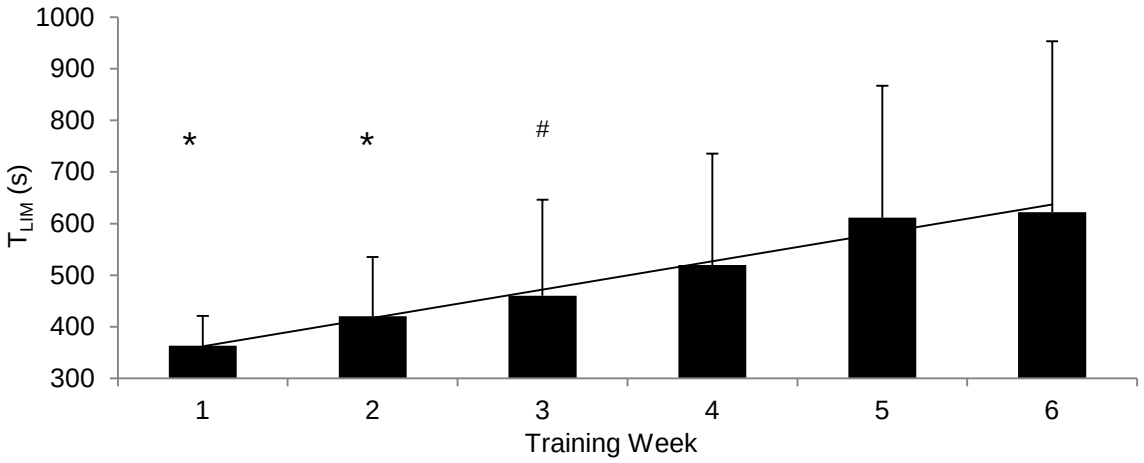

Figure 7.10 Mean ± SD performance time (T_{LIM}; session 2) throughout training. * Weeks 1 and 2 < than weeks 4, 5 and 6 ($P < 0.05$), # Week 3 < than weeks 5 and 6 ($P < 0.03$).

7.5.3 Power output

The greatest peak power output (PPO) within a training session for weeks 1 to 6 was recorded during the first 30 s (Wingate) sprint, regardless of the number of subsequent sprints. However, there was no difference over the 6 weeks ($P = 0.25$) with a mean (corrected) PPO for sprint 1 of 1176 ± 279 W (Figure 7.11). Overall mean PPO was 1056 ± 260 W. Regardless of the number of 30 s sprints, PPO reduced over time during each training session 3. Large effect sizes (partial η^2 values) were associated with these changes (Table 7.8). Interestingly the lowest PPO occurred on the final 30 s sprint for weeks 1, 2, 5 and 6 but on the penultimate sprint for weeks 3 and 4 (i.e. when training load was highest).

As for PPO, the greatest mean power output (MPO) for weeks 1 to 6 inclusive was recorded during the first 30 s (Wingate) sprint, regardless of number of subsequent repetitions. Similarly, there was no difference over the 6 weeks ($P = 0.24$) with a mean MPO of 630 ± 115 W (Figure 7.12). Overall mean MPO was 570 ± 113 W. As for PPO, MPO reduced over time during training session 3. Similarly, partial η^2 values demonstrate that

these changes were large (Table 7.8). With the exception of week 3 (penultimate) the lowest MPO for all weeks was recorded in the final sprint.

Table 7.8 P and partial η^2 values for differences in PPO and MPO across sprints during session 3

	Training Week #					
	1	**2**	**3**	**4**	**5**	**6**
PPO						
P	0.003	< 0.001	< 0.001	0.003	< 0.001	< 0.001
Partial η^2	0.57	0.69	0.65	0.63	0.61	0.73
MPO						
P	< 0.001	0.004	< 0.001	0.009	< 0.001	0.001
Partial η^2	0.82	0.64	0.66	0.54	0.81	0.63

Note: # The number of sprints for weeks 1 to 6 was 4, 5, 6, 6, 5, and 4, respectively.

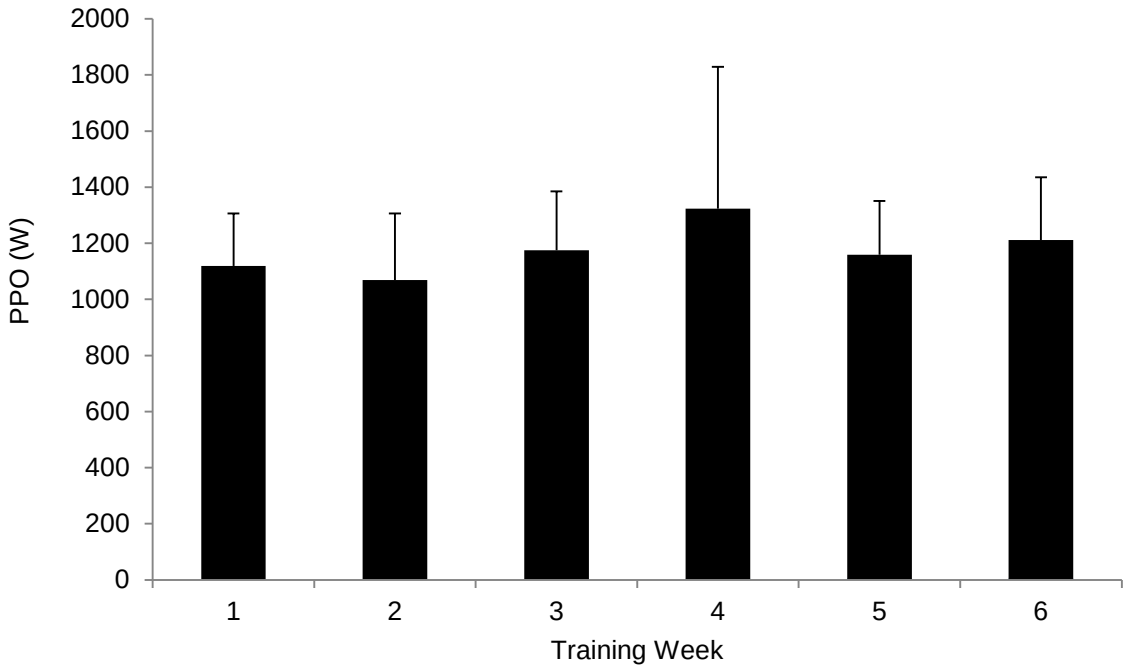

Figure 7.11 Peak power output (PPO) from first 30 s Wingate sprint for weeks 1 to 6

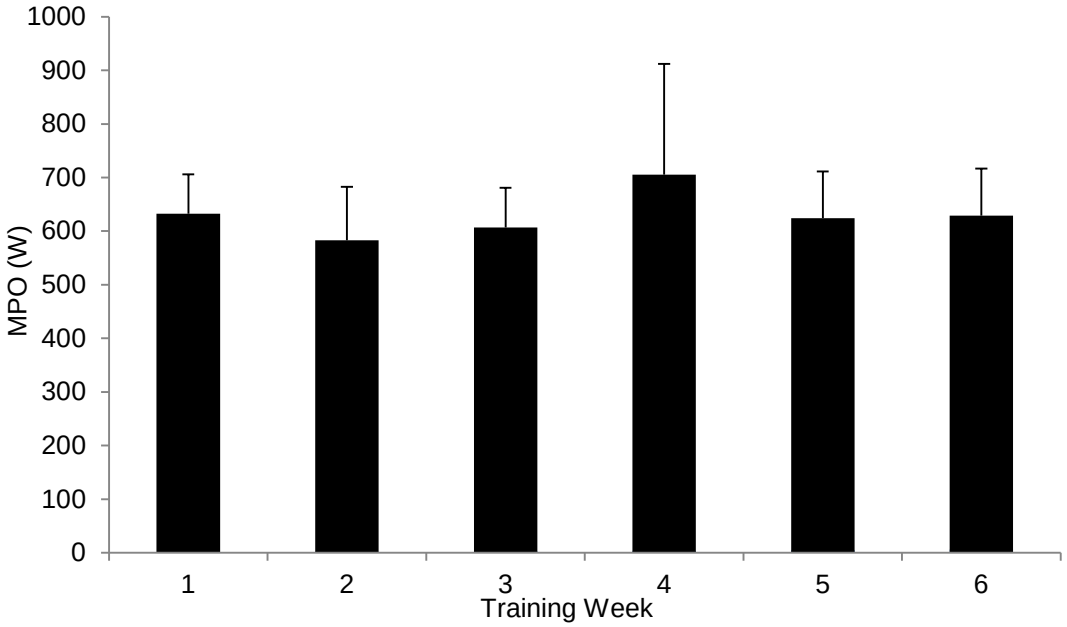

Figure 7.12 Mean power output (MPO) from first 30 s Wingate sprint for weeks 1 to 6

7.6 Discussion

This study evaluated the effects of 6 weeks high-intensity cycling training in non-cycling trained males on the efficacy of sodium bicarbonate ($NaHCO_3$) as an ergogenic aid. $NaHCO_3$ ingestion led to severe gastro-intestinal (GI) discomfort in 2 participants (1 x pre-training and 1 x post-training), something that is well reported in the literature evaluating the effects of $NaHCO_3$ on exercise performance (Cameron et al. 2010, Saunders et al. 2011). Indeed, Saunders et al. (2011) found that when participants who suffered GI distress (n=4/21) were removed from the analysis, $NaHCO_3$ was shown to enhance total work done by 5% whereas whole group data did not show a significant difference. Based on $NaHCO_3$ ingestion leading to obvious outliers (Figure 7.1) the majority of results were analysed for n=8. However, for illustrative purposes key comparisons between n=8 and n=10 are discussed where appropriate.

Before training (n=8), T_{LIM} was 10% greater with $NaHCO_3$ ingestion compared to PLA which is less than the 17% difference in exercise capacity between $NaHCO_3$ and PLA at

100% W_{PEAK} in study 2 (chapter 5). This response was observed despite no statistically significant difference (P = 0.28) in 100% W_{PEAK} between the present study (249 ± 34 W) and study 2 (228 ± 37 W). However, with an effect size of 0.58, ~ 3 times the smallest worthwhile effect (Hopkins 2004), it is likely there was a moderate difference in W_{PEAK} between studies. It is unclear as to whether this difference in W_{PEAK} contributed to the greater difference in T_{LIM} performance between $NaHCO_3$ compared to PLA at 100% W_{PEAK} in study 2. Regardless, both improvements in T_{LIM} are comfortably more than the 6% / 16 s daily variation in T_{LIM} reported in study 1 (chapter 4) and are thus likely to be biologically significant. More simply, $NaHCO_3$ demonstrated biologically important benefits at 100% W_{PEAK} in both study 2 and 4. After 6 weeks high intensity training post-training (n=8) T_{LIM} was no greater than daily variation for $NaHCO_3$ compared with PLA. Therefore at the group level, based on being greater than the recommended benefit to harm odds-ratio of > 66 and considering daily variation of T_{LIM} performance in our laboratory, $NaHCO_3$ ingestion would be recommended for T_{LIM} at 100% W_{PEAK} before but not after 6 weeks high-intensity training (Table 7.5., Hopkins 2007, Hopkins et al. 2009). Interestingly, when considering the n=10 population, pre-training T_{LIM} was 5% greater with $NaHCO_3$ than PLA and post-training T_{LIM} was 1% *lower* with $NaHCO_3$ than PLA. Although the absolute figures change quite dramatically after removing the participants who suffered extreme GI discomfort, there remains a general trend suggesting that the efficacy of $NaHCO_3$ is likely compromised with an improvement in training status (Tables 7.4 and 7.5).

It is important to acknowledge that some individual variation in T_{LIM} performance was observed between treatments pre and post-training although this was less marked than in studies 2 and 3. For example, there was no difference between treatments for pre-training T_{LIM} for 4 participants. However, post-training T_{LIM} was greater than daily variation after $NaHCO_3$ for 1 participant, greater after PLA in 2 participants with no difference between treatments in the final participant. In contrast for the remaining 4 participants, T_{LIM} was greater with $NaHCO_3$ both pre and post-training. Finally, pre-training 4/8 participants

improved T_{LIM} with $NaHCO_3$ supplementation in contrast to 5/8 post-training. However, it should be acknowledged that in the 5/8 participants whose pattern of exercise capacity was similar pre and post training, there was actually a mean reduction in T_{LIM} of -5% demonstrating that post-training, the efficacy of $NaHCO_3$ was reduced in this sub-group. The variation in T_{LIM} performance between treatments pre and post-training is likely to be, at least in part, related to differential training responses between individuals (Suzuki et al. 2004, Bishop et al. 2008). In summary, it is important to remember that group level data does not necessarily represent individual responses. Therefore, although group level data is in accordance with our original hypothesis an individualised approach should be considered in an applied setting.

As high-intensity exercise progresses the intracellular buffering capacity is eventually exceeded stimulating the extracellular buffering mechanisms to modulate increases in lactate and H^+ which diffuse into the blood (Matson and Tran 1993). Carnosine is an intracellular buffer which has been suggested to play an important role in the homeostasis of muscle cells during high-intensity exercise (Derave et al. 2010). Indeed, individuals who have undertaken repetitive high-intensity training are reported to possess greater levels of intracellular carnosine than those who have undertaken extended endurance training or untrained healthy controls (Parkhouse et al. 1985). Furthermore, sprint training in previously untrained but healthy males, similar to the present study, reported a mean increase in carnosine of 113% (range: 37% to 241%) which the authors implicated as key to greater PO during subsequent WAnT sprinting (Suzuki et al. 2004). It appears likely that intramuscular carnosine content increased as much, if not more, as reported by Suzuki et al. (2004) due to the greater specificity and training load employed in the present study. Therefore, it is plausible that increases in intracellular carnosine have played a direct role in reducing the efficacy of $NaHCO_3$. After training induced augmented intracellular buffering, during T_{LIM} there would be less diffusion of H^+ into the extracellular fluid and thus less extracellular buffering required. Indeed, in the present study although post-exercise pH (7.27 ± 0.07 vs.

7.26 ± 0.06, ES = 0.2), BE (-11.0 ± 3.9 vs. -12.1 ± 4.2 mmol.l^{-1}, ES = 0.3) and [HCO_3^-] (14.6 ± 2.8 vs. 13.5 ± 3.4 mmol.l^{-1}, ES = 0.4) were not reported to be statistically different post versus pre-training respectively, effect size analysis suggest H^+ buffering/regulation was augmented during exercise. Similarly, pH (7.30 ± 0.07 vs. 7.28 ± 0.06, ES = 0.3), BE (-9.4 ± 4.4 vs. -11.5 ± 3.5 mmol.l^{-1}, ES = 0.5) and [HCO_3^-] (15.7± 3.3 vs. 13.6 ± 2.9 mmol.l^{-1}, ES = 0.7) were all greater post-training compared to pre-training 5-mins post exercise. Therefore, although not measured in the present study it would appear likely that training induced increases in intramuscular carnosine, to at least some extent, have reduced the demand for extracellular buffering of H^+ during exercise and might have contributed to better recovery of acid-base homeostasis post-exercise.

Although training induced augmented levels of carnosine might reduce the need for extracellular buffering during T_{LIM} it might not be the only adaptive mechanism providing H^+ regulation post-training. Juel (1998) reported that activity of the Na^+/H^+ exchanger in rat muscle was elevated after 6 weeks of high-intensity treadmill training but unchanged after endurance training. Therefore, in the present study it is possible that training induced increases in the Na^+/H^+ exchanger facilitated greater H^+ regulation (i.e. clearance) post-exercise. However, Harmer et al. (2000) reported that 7 weeks sprint training in humans, similar to the present study, might reduce H^+ production and/or removal during exercise. However, in contrast to Harmer et al. (2000) the present study did not compare physiological responses post-training at the same relative level to that of pre-training and therefore, this might have masked improved H^+ regulation until during post-exercise recovery as demonstrated by better recovery of acid-base homeostasis 5 mins post-exercise post-training. In summary, although increases in intracellular carnosine likely contributed to the reduction in efficacy of $NaHCO_3$, improved H^+ buffering and/or regulation might also occur due to up-regulation of the Na^+/H^+ exchanger and thus also contribute to the reduction in efficacy of $NaHCO_3$.

The difference in BLa between $NaHCO_3$ and PLA at the end of T_{LIM} both pre (17.0 ± 2.9 vs. 12.5 ± 1.6 mmol.l^{-1}) and post-training (13.4 ± 2.6 vs. 10.8 ± 2.2 mmol.l^{-1}) is consistent with previous research which proposed that performance improvement might not occur unless a difference of > 2 mmol.l^{-1} BLa is observed when using $NaHCO_3$ administration (Ibanez et al 1995). However, similar to results for T_{LIM} at 110% and 120% W_{PEAK} in study 2 (chapter 5), despite BLa being ≥ 2 mmol.l^{-1} at the end of exercise post-training, group level T_{LIM} at 100% W_{PEAK} after $NaHCO_3$ ingestion was no greater than daily variation. Moreover, the significant correlation between the difference in BLa and the % difference in T_{LIM} between treatments at the end of exercise pre-training was not observed post-training. Such variability is in accordance with data from study 2 (chapter 5) where a significant correlation was reported for the difference in T_{LIM} and difference in BLa between treatments at the end of exercise at 110% W_{PEAK} but not at 100% and 120% W_{PEAK}. Data from the present study provide further evidence that differential responses in BLa are not necessarily reliable indicators of differences in T_{LIM} performance when comparing $NaHCO_3$ and PLA.

In the present study BLa was lower post-training at the end of T_{LIM} (12.1 ± 2.7 vs. 14.7 ± 3.3 mmol.l^{-1}) and 5-mins post (10.7 ± 2.8 vs. 13.0 ± 3.0 mmol.l^{-1}). Although not measured directly in the present study it seems plausible that, to at least some extent, enhanced lactate handling has taken place during post-training T_{LIM}, most likely due to training induced up-regulation of monocarboxylate transporters (MCT) isoforms MCT1 and/or MCT4. Indeed, MCT1 and MCT4, appear crucial to blood lactate transport through their facilitative role in a cell-to-cell lactate shuttle (Thomas et al. 2005). High-intensity training has been shown to increase both MCT1 and MCT4 expression in rats although this was specific to soleus muscle. Interestingly, increases in MCT4 were greatest when $NaHCO_3$ was consumed before training (Thomas et al. 2007). The effects of high-intensity training on MCT1 and MCT4 expression in humans have also been examined (Pilegaard et al. 1999, Bishop et al. 2008). Pilegaard et al. (1999) reported that MCT1 and MCT4 were 70% and 33% higher, respectively, in trained compared to untrained muscle after high-

intensity knee extensor exercise. Thomas et al. (2005) found that endurance trained participants had significantly greater MCT1 (49%) than less trained participants. Moreover, MCT1 expression was negatively correlated with BLa concentration after supramaximal exercise suggesting MCT1 might augment tolerance to fatigue. Although MCT4 was not reported to be statistically different between groups (29% increase for trained compared to untrained individuals), there was an extremely large effect size (not published) of 2.5 for differences in MCT4 (3.5 for MCT1) suggesting differences in MCT4 might also predispose trained individuals to higher levels of fatigue tolerance from intracellular mechanisms. Indeed MCT4 expression was also negatively correlated with BLa concentration after supramaximal exercise (Thomas et al. 2005). It should also be acknowledged that Bishop et al. (2008) reported no change in MCT1 or MCT4 concentration after high-intensity cycling interval training. Nevertheless, the authors reported changes (expressed relative to pre-training values) of 96 ± 12% for MCT1 and 119 ± 21% for MCT4 demonstrating significant individual variation, especially in MCT4. Moreover, the timing of the biopsy (immediately post-exercise) and exercise protocol might have influenced these results (Bishop et al. 2007, Bishop et al. 2008). Interestingly, Juel (2006) suggests that 6-8 weeks high-intensity training is sufficient to substantially increase sarcolemma bound proteins such as MCT1 and MCT4, with further training having no effect. Nevertheless, despite probable increases in both MCT1 and MCT4 and hence lower BLa post-exercise and 5 minutes post-exercise compared to pre-training (and > 2 $mmol.l^{-1}$ for $NaHCO_3$ compared to PLA) at a group level $NaHCO_3$ did not enhance T_{LIM} beyond daily variation post-training. These data demonstrate that despite enhanced lactate handling post-training differential responses in BLa remain a poor predictor of differences in T_{LIM} performance when comparing $NaHCO_3$ and PLA. It should be remembered that MCTs also play a crucial role in pH regulation during high-intensity exercise. By facilitating lactate-H^+ co-transport across the plasma membrane, MCTs reduce intracellular acid-base stress and theoretically provide more opportunity for extracellular H^+ buffering. With augmented buffering capacity after $NaHCO_3$ ingestion this might prevent deleterious changes in pH for longer and thus enhance exercise capacity. However, at a

group level this was not evident in the present study. This might be related to differential responses to training, not only in MCTs (Bishop et al. 2008) and carnosine (Suzuki et al. 2004) but possibly differences in specific isoforms of carbonic anhydrase (CAIV and CAXIV) which facilitates the hydration/dehydration reaction between CO_2, HCO_3^-, and H^+ in vivo (Messonier et al. 2007). Moreover, with augmented intracellular carnosine and/or up-regulation of the Na^+/H^+ exchanger likely to play key roles, it is not clear what role, if any, augmented MCTs affect the efficacy of $NaHCO_3$.

After 6 weeks high-intensity training participants improved $\dot{V}O_{2PEAK}$ by 14 ± 11 % (46 to 52 $ml.kg^{-1}.min^{-1}$). Therefore, although in absolute terms there were no changes in $\dot{V}O_2$ at the end of T_{LIM} pre and post-training it would appear that participants had become more economical users of oxygen. This is supported by McKenna et al. (1997) who suggested that the major energetic adaptation of repeated WAnT training with limited recovery appears to be enhanced aerobic metabolism. In accordance with McKenna et al. (1997) MacDougall et al. (1998) found that 7 weeks of repeated sprint training similar to the present study increased $\dot{V}O_{2MAX}$ by ~ 7%. Moreover, oxidative enzymes citrate synthase and succinate dehydrogenase increased by ~ 36% and ~ 65%, respectively and glycolytic enzymes PFK and hexokinase increased by ~ 49% and ~ 56%, respectively. Indeed, MacDougall et al. (1998) suggest that increased activity of PFK might accelerate glycolytic flux during maximum sprint efforts which might explain, at least in part, why participants in the present study improved T_{LIM} during training and $\dot{V}O_{2PEAK}$ post-training. Indeed, in the context of the present study training induced increases in oxidative enzymes are likely to have facilitated greater intracellular lactate oxidation during post-training T_{LIM}. Therefore, in addition to up-regulation of MCT1 and/or MCT4 this supports the lower BLa values recorded post-training. In contrast, Suzuki et al. (2004) reported no change in $\dot{V}O_{2PEAK}$ after repeated high-intensity sprint training which is most likely due to the differences in training protocols adopted. Combined, these results would seem to suggest that there is a threshold of both training stimulus and recovery time required to stimulate oxidative and glycolytic enzymatic changes

during training (MacDougall et al. 1998, Suzuki et al. 2004, Burgomaster et al. 2005). It appears highly likely that the training stimulus in the present study was sufficient to stimulate similar positive enzymatic changes although the precise extent of such changes is unclear. Similarly for augmented MCT abundance, it is not clear what role that, if any, enzymatic changes play in the reduction in efficacy of $NaHCO_3$ post-training.

Although overall AD (i.e. over all trials pre and post training) was low (1.3 ± 1.8), 6% (8 out of 128) of AD ratings were ≥ 6 units (range 6.0 to 8.0). Interestingly, the highest rating of AD (8.0) was noted prior to $NaHCO_3$ ingestion which decreased at 30 mins post ingestion (6.0), pre-exercise (6.0) and at the end of exercise (0.0). It is unclear why AD was so high before $NaHCO_3$ ingestion for this participant. However, despite such high ratings of AD prior to exercise, T_{LIM} (post-training) was 8% (22 s) greater for $NaHCO_3$ compared to PLA in this participant. Similarly, although overall GF was low (2.1 ± 1.9), 9% (11 out of 128) of GF ratings were ≥ 6.0 units (range 6.0 to 7.0). This was largely due to a different participant who registered a 5.0 or 6.0 for GF at every time point regardless of treatment. Interestingly, the participant who registered a GF score of 7.0 units 30 mins prior to both $NaHCO_3$ trials improved T_{LIM} compared to PLA by 22% and 25%, pre and post-training, respectively. Similar to previous research (Price and Simons 2010) and data from study 2 (chapter 5), GI distress does not always negatively influence performance. However, one limitation of the present study is that the AD and GF scales have not been experimentally validated. Therefore, it is plausible that a more sensitive tool might present slightly different results.

Although similar after 1 min, 3 mins and at the end of exercise $NaHCO_3$ attenuated RPE_L to a greater extent than PLA after 2 mins during T_{LIM}, pre-training. In contrast there was no difference in RPE_L at any time point post-training. The difference in RPE_L after 2 mins is almost identical to that reported for 100% T_{LIM} in study 2 (chapter 5) where after 1 and 2 mins RPE_L was attenuated. This suggests that $NaHCO_3$ plays some contributory role in modulating RPE_L during the early stages of high-intensity exercise at 100% W_{PEAK}. An

explanatory mechanism is yet to be reported but it could be that pre-exercise alkalosis attenuates the stress response (i.e. specific heat shock proteins such as HSP72) and subsequent afferent neural feedback during exercise and concomitantly facilitates the attenuation of RPE_L. Peart et al. (2011) found that 0.3 $g.kg^{-1}$ $NaHCO_3$ significantly attenuated the HSP72 response 30 mins after exercise when compared with NaCl ingestion (increases of 5% and 42% of HSP72, respectively). Nevertheless, no performance benefit was observed although the 4 mins 'all-out' protocol used is likely to have been a contributory factor (Peart et al. 2011, Vanhatalo et al. 2010). It is unclear why modulation of RPE_L was observed pre but not post-training. It could be that training induced changes somehow dampens any $NaHCO_3$-HSP72 modulation of RPE_L. Contrastingly $NaHCO_3$ might attenuate HSP72 post-training to a similar or even larger extent/rate post-training but inhibition of and/or superseded afferent feedback signals prevent this translating into a reduction in RPE_L. However, this is speculative and further research on the role of $NaHCO_3$ ingestion in modulation of RPE_L is warranted.

Pre-training RPE_O for T_{LIM} was lower when compared to post-training for all time points and for both treatments. However, it is important to remember that post-training participants were cycling at a greater absolute W_{PEAK} which is likely to contribute to increased effort perception post-training (pre-training T_{LIM} was 89 ± 8% of post-training T_{LIM}; range: 74% to 98%). Ideally, participants would have repeated the T_{LIM} experimental trials at the pre-training 100% W_{PEAK} to allow further comparison. Unfortunately, due to time and logistics this was not possible. Our observation that RPE_L was greater than RPE_O at each time point is consistent with previous research (Hetzler et al. 1991) demonstrating that RPE_L comprises a greater proportion of overall effort perception in cycle exercise in previously untrained males (Hetzler et al. 1991, Hampson et al. 2001).

In the present study $NaHCO_3$ treatment resulted in an overall increase in HR of 5 bpm^{-1} compared to PLA. Although there was no difference pre-ingestion, pre-exercise HR

increased by 7 bpm^{-1} after $NaHCO_3$ ingestion compared to PLA. Heart rate remained similarly elevated at volitional exhaustion and 5 mins post-exercise. The effect of $NaHCO_3$ ingestion on HR pre-exercise and at volitional exhaustion in the present study is almost identical to that reported in study 2 (chapter 5). The mechanism for why $NaHCO_3$ ingestion causes elevation of HR prior to exercise and why HR remains elevated during and after exercise is as yet unconfirmed. This might be related to differential responses during digestion which lead to increased blood flow (presumably predominantly to the GI tract) and HR after $NaHCO_3$ ingestion compared to PLA. Anecdotal evidence suggests there are more incidents of diarrhoea and general toilet visits after $NaHCO_3$ ingestion which might increase HR through more pre-exercise general low level activity (i.e. no significant increase in BLa). Alternatively, there might be some mild dehydration for those who suffer diarrhoea despite being able to drink water *ad libitum*. However, such suggestions are speculative.

One of the principles of successful physical training is specificity (Baechle and Earle 2008). Based on this and on the results from study 2 (chapter 5), we included 100% W_{PEAK} T_{LIM} (session 2) as part of the training program. Mean T_{LIM} improved by 71% over 6 weeks. However the mean improvement based on participants longest compared to shortest T_{LIM} over the 6 weeks was 149% (range: 59% to 290%) due to 5 out of 8 participants recording their highest T_{LIM} in week 5 rather than week 6. This might be due to general transient training fatigue between week 5 and 6. Indeed, it appears unlikely that overtraining played any significant role as participants recorded a mean increase in W_{PEAK} of 12 ± 7% after completing training. The large (149%) increase in T_{LIM} during training is similar to the 123% improvement in 100% W_{PEAK} T_{LIM} recorded by Edge, Bishop, and Goodman (2006). It seems logical that incorporation of specific training in the present study is, at least in part, why improvements in 100% T_{LIM} in the present study were higher than recorded by Edge, Bishop, and Goodman (2006).

In summary, 6 weeks high-intensity cycling training reduces the effectiveness of $NaHCO_3$ in enhancing maximal cycling capacity in previously non-cycling trained males. More research is required to elucidate the mechanisms underpinning this change in efficacy. The most plausible explanations include; training induced changes in intracellular buffering capacity, with augmented carnosine likely to play some role and up-regulation of the Na^+/H^+ exchanger. Further research using the biopsy technique to ratify these results (including analysing changes in carnosine, the Na^+/H^+ exchanger and possibly MCT1/4) is warranted as is a cross sectional study (i.e. variety of basal training statuses) evaluating the effects of an increase in training status on the efficacy of $NaHCO_3$ ingestion. Particular attention should focus on the impact of effort perception during exercise and how each of the above mechanisms might attenuate RPE_L during high-intensity exercise. Finally, although group level data is in accordance with our original hypothesis an individualised approach should be considered in an applied setting.

Chapter 8 – General conclusion

8.1 Summary

By evaluating the efficacy of $NaHCO_3$ as an ergogenic aid from both whole body (*in vivo*) and isolated muscle (*in vitro*) perspectives this thesis has presented new and in-depth evidence in relation to the effects of $NaHCO_3$ on whole body and isolated muscle performance. The following is a summary of the unique and key findings of this thesis:

1. Before experimental data collection, two familiarisation sessions are required to adequately familiarise human participants undertaking cycling exercise capacity tests **(chapter 4)**.

2. $NaHCO_3$ improved cycling exercise capacity (T_{LIM}) in humans at 100% W_{PEAK} but not 110% or 120% W_{PEAK}. By evaluating the efficacy of $NaHCO_3$ over this range of exercise intensities in the same population we are the first to demonstrate that the 'responder', 'non-responder' classification for $NaHCO_3$ ingestion is too simplistic **(chapter 5)**.

3. $NaHCO_3$ had a significantly greater effect when T_{LIM} was longer than 5 mins whereas no difference between treatments was observed when T_{LIM} was less than 5 mins for continuous work tests **(chapter 5)**. This has challenged the traditional 1 to 7 minute window for exercise duration proposed for when $NaHCO_3$ might be effective.

4. $NaHCO_3$ ingestion attenuated RPE_L compared to PLA after 1 min and 2 mins of exercise during the 100% W_{PEAK} trial only **(chapter 5)**. The attenuation of RPE_L after 2 mins after $NaHCO_3$ ingestion was also found to be a repeatable response in non-cycling trained males **(chapter 7)**.

5. In contrast to Ibanez et al. (1995) we have demonstrated that a difference of ≥ 2 mmol.l^{-1} in BLa at the end of exercise of does not necessarily correlate to greater exercise capacity after $NaHCO_3$ ingestion **(chapters 5 and 7)**.

6. In isolated mouse muscle acute PO was on average 7.0 % greater for $NaHCO_3$ treated EDL muscles and 3.6 % greater for $NaHCO_3$ treated SOL muscles when compared to CON. The acute effects of $NaHCO_3$ on EDL were significantly greater than on SOL **(chapter 6)**.

7. Increases in acute PO in isolated mouse muscle were due to greater force production throughout shortening. These results present the best indication to date that $NaHCO_3$ has direct peripheral effects on mammalian skeletal muscle resulting in increased acute power output **(chapter 6)**.

8. $NaHCO_3$ treatment did not alter the pattern of fatigue during dynamic work loop simulation in isolated mouse muscle. However the fatigability of muscle performance was variable suggesting, that there might be inter-individual differences in response to $NaHCO_3$ supplementation at the muscle level **(chapter 6)**.

9. During 60 mins recovery $NaHCO_3$ treated muscle demonstrated a poorer mean recovery than CON after 10 minutes for EDL only. Overall, recovery of PO was significantly greater in SOL (91 ± 8%) compared to EDL (59 ± 26 %). Importantly, both EDL (~ 80%) and SOL (~ 90%) muscles recovered almost completely within one hour of fatiguing exercise, regardless of treatment **(chapter 6)**.

10. An improvement in training status after 6 weeks high-intensity cycling training in non-cycling trained males nullifies the efficacy of sodium bicarbonate ($NaHCO_3$) as an ergogenic aid **(chapter 7)**.

11. $NaHCO_3$ ingestion attenuated RPE_L compared to PLA after 2 mins of exercise during 100% W_{PEAK} cycling. However, after 6 weeks high-intensity cycling training there were no differences in RPE_L between treatments **(chapter 7)**.

8.2 Overall efficacy of $NaHCO_3$

We have examined the efficacy of $NaHCO_3$ as an ergogenic aid in humans at different exercise intensities (chapter 5) and after an improvement in training status (chapter 7). In study 2 (chapter 5) we demonstrated that T_{LIM} for all exercise intensities (n=10) was 9% greater for $NaHCO_3$ compared to CON and in study 4 (chapter 7) we demonstrated that 6 weeks high intensity cycling training in untrained males nullifies the ergogenic benefit observed pre-training. Figure 8.1 presents the overall efficacy (n=18) of $NaHCO_3$ as an ergogenic aid for all trials completed at 100% W_{PEAK} in this thesis inclusive (n=52) and exclusive (n=36) of 100% W_{PEAK} post-training trials (i.e. excluding training effects).

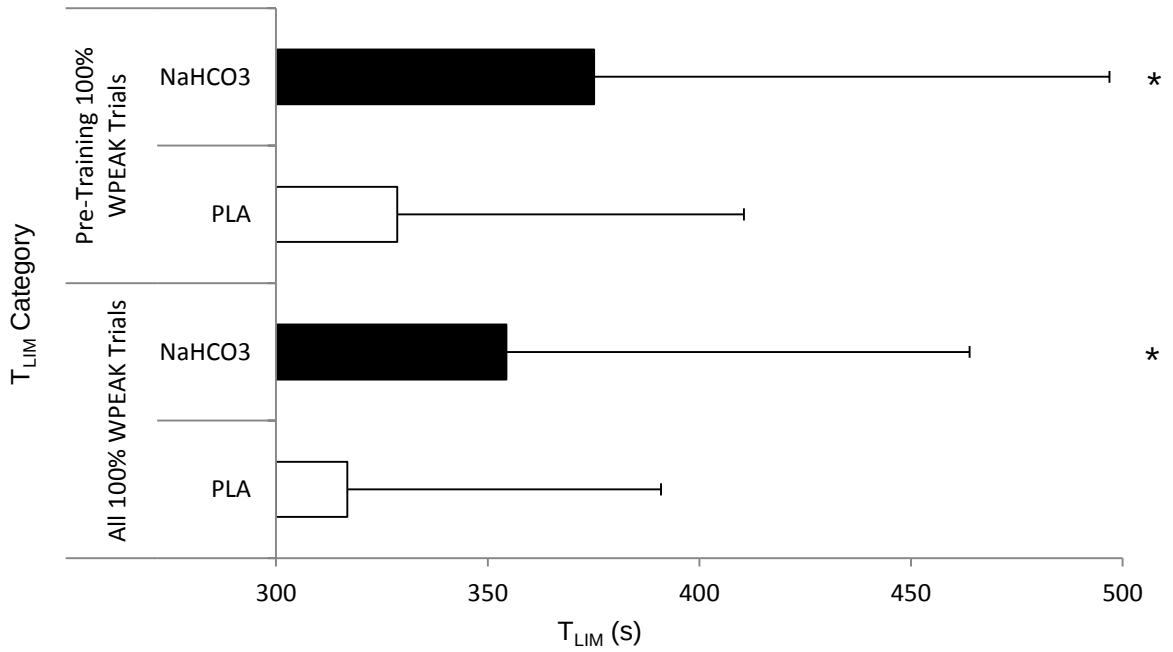

Figure 8.1 The overall efficacy of $NaHCO_3$ compared to PLA at 100% W_{PEAK} exclusive (top) and inclusive (bottom) of post-training T_{LIM} trials. * $NaHCO_3$ > PLA (P = 0.002; Wilcoxon test)

The overall efficacy of $NaHCO_3$ compared to PLA at 100% W_{PEAK} inclusive of all trials was 38 s / 12% (ES = 0.4, P = 0.002). When considering 100% W_{PEAK} exclusive of post-training trials efficacy of $NaHCO_3$ increased to 47 s / 14% (ES = 0.4, P = 0.002, Figure 8.1). This further demonstrates that 6 weeks high intensity training significantly reduced the efficacy of $NaHCO_3$.

In summary, $NaHCO_3$ is an effective ergogenic aid, most likely observed at 100% W_{PEAK} in non-cycling specific trained healthy males (chapter 5). However, the efficacy of $NaHCO_3$ is variable at other exercise intensities (chapter 5) and likely diminishes after an improvement in training status (chapter 7). Interestingly, there was no difference in T_{LIM} at 100% W_{PEAK} in study 2 (chapter 5) compared to pre-training T_{LIM} at 100% W_{PEAK} study 4 (chapter 7; 18 s, 5%, P = 0.93, ES = 0.1) demonstrating repeatability at 100% W_{PEAK} in untrained males after $NaHCO_3$ ingestion.

8.2.1 Responders / non responders classification

As described in section 8.1 and study 2 (chapter 5) the responder / non-responder classification for human performance after $NaHCO_3$ ingestion is too simplistic. Further support for this can be found in study 4 (chapter 7) when evaluating data from participants who completed both $NaHCO_3$ studies. We found that the only participant in study 2 who improved T_{LIM} after $NaHCO_3$ ingestion at all exercise intensities did not improve T_{LIM} at 100% W_{PEAK} pre or post 6 weeks high intensity training (study 4). In fact, post-training T_{LIM} was 17% (52 s) lower after $NaHCO_3$ compared to PLA. A difference in training status appears unlikely for the change in efficacy of $NaHCO_3$ as the initial $\dot{V}O_{2PEAK}$ of this participant was very similar between studies (40 and 44 $ml.kg^{-1}.min^{-1}$ for studies 2 and 4, respectively). Additionally, in examining data from the three other participants who completed both studies there is no evidence to support the responder / non-responder classification. Indeed, one of the participants who was removed from the analysis in study 4, due to GI distress and

subsequent dramatic performance reduction, improved T_{LIM} at 100% W_{PEAK} by 26% (73 s) and by 16% (21 s) at 120% W_{PEAK} after $NaHCO_3$ ingestion. There was no difference at 110% W_{PEAK} (- 9 s / - 4%; study 2, chapter 5). Interestingly, there was considerable inter-individual variation noted for isolated EDL and SOL during fatiguing exercise. Therefore, in contrast to whole body performance, the responder / non-responder classification for isolated mouse EDL and SOL muscle undergoing cyclical length changes might be appropriate.

8.3 Perceptual responses

8.3.1 Ratings of perceived exertion (RPE)

We have demonstrated that $NaHCO_3$ ingestion attenuates RPE_L during the early stages of high intensity exercise at 100% W_{PEAK} compared to PLA in non-cycling trained males. Specifically, RPE_L was attenuated after 2 mins exercise compared to PLA (chapters 5 and 7). Interestingly, the attenuation of RPE_L after 2 mins exercise was not observed after an improvement in training status. This data demonstrates that in non-cycling trained males the attenuation of RPE_L at 100% W_{PEAK} is a repeatable perceptual response. However, this response does not occur after an improvement in training status, the mechanisms for which are, at present, unclear. It is possible that RPE_L was attenuated at 100% W_{PEAK} (and not at 110% and 120% W_{PEAK}) because the associated biochemical and physiological changes occurred at a rate that produced neuro-physiological feedback that facilitated attenuated RPE_L. An explanatory mechanism is yet to be fully elucidated but might be linked to the attenuation of perceived exertion by endogenous opioids (Sgherza et al. 2002), which in itself is likely driven by exercise intensity. Indeed Sgherza et al. (2002) reported that an exercise intensity threshold of ~ 60-75% $\dot{V}O_{2MAX}$ is required to stimulate the release of endogenous opioids. Therefore, it is plausible that the same holds true with increasing exercise intensity. In other words, although endogenous opioids might still be released beyond a certain exercise intensity threshold, for example ~ 100% $\dot{V}O_{2MAX}$, above this threshold the associated changes in neuro-physiological feedback might prevent the

attenuation of RPE_L. Similarly it could be that pre-exercise alkalosis attenuates the stress response (i.e. specific heat shock proteins such as HSP72) during exercise (Peart et al. 2011) and concomitantly facilitates the attenuation of RPE_L. The reason(s) why RPE_L was not attenuated after $NaHCO_3$ ingestion during T_{LIM} at 100% W_{PEAK} after an improvement in training status are unclear. However, this might be due to general increased tolerance of discomfort during exercise which dampens any pre-training benefits of $NaHCO_3$ on RPE_L responses. Clearly, more research on the effects of $NaHCO_3$ and RPE_L responses is warranted.

8.3.2 Abdominal discomfort (AD) and gut fullness (GF)

Reports of GI discomfort after $NaHCO_3$ ingestion are well reported in the literature (Cameron et al. 2010, Price and Simons 2010, Carr et al. 2011). Based on an eleven point (0-10) Likert scale (sections 10.1 and 10.2), overall ratings (n=18; n=10 from study 2 plus n=8 from study 4) of AD after $NaHCO_3$ ingestion 30 mins before exercise and immediately before exercise were 3.0 ± 2.3 and 2.5 ± 2.1, respectively. Similarly, overall ratings (n=18) for GF 30 mins before exercise and immediately before exercise were 2.9 ± 2.1 and 2.5 ± 1.9, respectively. This summary data supports the individual data reported in study 2 (chapter 5) and study 4 (chapter 7) that ratings of AD and GF were mild after $NaHCO_3$ ingestion. Nonetheless, as reported in study 4 two participants suffered such severe GI discomfort that their cycling performance was detrimentally affected. By removing these participants from the final analysis the overall view of the efficacy of $NaHCO_3$ changed quite dramatically. A change in efficacy of $NaHCO_3$ has been observed previously when participants who suffer severe GI distress are removed from the overall analysis (Saunders et al. 2011). Nevertheless the trend for $NaHCO_3$ being effective before but not after an improvement in training status remained (Table 7.5).

There were medium to large positive correlations (both 0.70) for the difference in T_{LIM} between $NaHCO_3$ and PLA and the absolute load of $NaHCO_3$ consumed both pre and post-training when analysing n=8 (study 4, chapter 7). However, this relationship disappeared for n=10 (Table 7.2). Based on the premise that heavier participants ingest a larger absolute load and appeared to suffer greater AD (the body mass of the two participants that suffered extreme GI distress (chapter 7) were 15% and 35% higher than the mean cohort body mass of 81 kg) we explored whether the absolute load of $NaHCO_3$ (i.e. effect of body mass) was related to AD and GF for n=18 and n=20 (i.e. with and without the participants who were removed from the analysis in study 4). For n=18 the correlation between $NaHCO_3$ load and AD pre-exercise was low and not significant ($\rho = 0.25$, $P = 0.099$). For n=18 the correlation between absolute $NaHCO_3$ load and AD 30 mins pre-exercise was also low but approached significance ($\rho = 0.28$, $P = 0.064$). In contrast, for n=20 there was a higher (albeit still low) and significant correlation ($\rho = 0.35$, $P = 0.012$) between absolute $NaHCO_3$ load and AD pre-exercise (Figure 8.2).

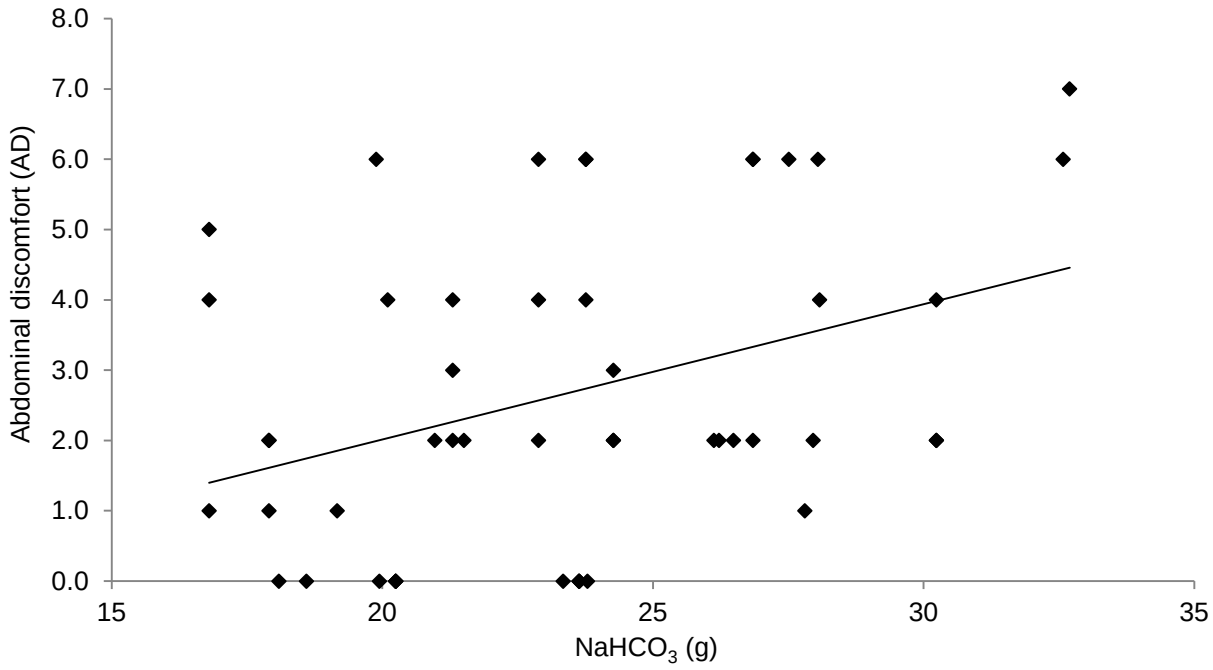

Figure 8.2 Correlation between pre-exercise abdominal discomfort (AD) and absolute $NaHCO_3$ load (g) (n=20)

For n=20 the correlation between absolute $NaHCO_3$ load and AD 30 mins pre-exercise was also low and approached significance (ρ = 0.28, P = 0.052). Therefore, higher AD appears to be related to higher body mass which might subsequently impact on T_{LIM} (Table 7.2). No significant correlations between $NaHCO_3$ and GF were observed for n=18 or n=20.

Based on the premise that AD appears more likely in heavier individuals, we evaluated the differences in AD from participants under/equal to 78 kg and participants over 78 kg (78 kg is mean human body mass for $NaHCO_3$ studies) for n=18 and n=20. When comparing participants under/equal to 78 kg and participants over 78 kg, there were no significant differences in AD 30 mins pre-exercise or pre-exercise for n=18 (P = 0.15, P = 0.25) or n=20 (P = 0.13, P = 0.09), respectively, However effect sizes of 0.5 and 0.5 (n=20) and 0.4 and 0.3 (n=18) for differences in AD 30 mins pre-exercise and pre-exercise, respectively, suggest there was a small effect of body mass increasing AD at both time intervals for those participants with more than 78 kg body mass. Therefore for individuals over ~ 78 kg a staggered or chronic loading regime rather than an acute dose might be beneficial in reducing GI discomfort (section 2.3.10) and potentially increase the chance of improving T_{LIM} (Table 7.2). In contrast, for those with a body mass of less than 78 kg it appears a dose of 0.3 $g.kg^{-1}$ body mass of $NaHCO_3$ (up to a threshold of ~ 23 g) is likely to minimise GI discomfort and increase probability of enhancing T_{LIM} at 100% W_{PEAK}.

Interestingly, one of the participants who was removed from the analysis in study 4 (chapter 7) had also previously completed study 2 (chapter 5). This participant reported greater AD in study 4 than study 2 (6.2 ± 1.2 and 2.7 ± 1.1, respectively) where in study 2 T_{LIM} improved by 28% at 100% W_{PEAK} and 16% at 120% W_{PEAK}. The greater ratings of AD were associated with an increase in body mass of ~ 8% between study 2 and 4. However, as the body mass of this participant was more than 100 kg on both occasions it is not clear why AD was reported to be so different between studies. It is possible that the participant

became more comfortable in reporting significant AD in study 4 compared to study 2 although this would not explain differences in performance between studies. It is also plausible that the threshold of absolute $NaHCO_3$ which increases the probability of AD is higher than ~ 23 g in some individuals.

It is possible that over time participants might develop improved tolerance for acute $NaHCO_3$ ingestion. In study 2 (chapter 5) we observed that the one participant who did not improve at 100% W_{PEAK} (-10% with $NaHCO_3$) suffered from GI distress in all $NaHCO_3$ trials. However, GI distress did not prevent improvement in exercise capacity at 120% W_{PEAK} (+16%) which occurred during the last trial and was accompanied by lower AD and GF ratings than reported at 100% and 110% W_{PEAK}. Additionally, one participant who improved only at 100% W_{PEAK} (+9%) did so in the last $NaHCO_3$ trial. Despite recording mid-high AD (6.0) after 30 mins, in line with the 110% and 120% $NaHCO_3$ trials (8.0 and 6.0), AD dropped substantially pre-exercise (2.0 vs. 6.0). Such results suggest that over time improved GI tolerance to acute $NaHCO_3$ ingestion might have contributed to improvements in exercise capacity in those individuals.

It is important to acknowledge that GI distress does not always negatively impact performance (Price and Simons 2010). As reported in study 2 (chapter 5) one participant who reported mid-high (6.0) AD 30 mins pre-exercise and immediately pre-exercise had the highest increase in T_{LIM} (+38%) at 100% W_{PEAK} with $NaHCO_3$. In study 4 (chapter 7), the highest rating of AD (8.0) was noted prior to $NaHCO_3$ ingestion which decreased at 30 mins post ingestion (6.0), pre-exercise (6.0) and at end of exercise (0). Although it is unclear why AD was so high before $NaHCO_3$ ingestion for this participant, T_{LIM} (post-training) was 8% (22 s) greater for $NaHCO_3$ compared to PLA. Furthermore, one participant who vomited ~ 30 mins after $NaHCO_3$ ingestion before their post-training 100% W_{PEAK} trial improved T_{LIM} by 11% compared to PLA. This was despite AD ratings of 6.0, 30 mins prior, immediately prior and immediately post-exercise. It should be noted that this participant was asked on multiple

occasions whether he wanted to continue and was insistent on completing the trial. The increased T_{LIM} performance with $NaHCO_3$ in this participant was consistent with pre-training performance (+21%) where AD was lower (4.0) but remained at this level 30 mins prior, immediately prior and immediately post-exercise. It should also be pointed out that a decrement in performance with $NaHCO_3$ can also occur without any associated GI discomfort. A different participant reported having no AD or GF during any trials (i.e. ratings of 0) but T_{LIM} for $NaHCO_3$ was -6% and -19% compared to PLA pre and post-training, respectively. In summary, there is no consistent pattern between the efficacy of $NaHCO_3$ and ratings of AD and GF.

8.3.3 Relationship between abdominal discomfort (AD) and RPE_L

When considering n=18, there were moderate significant correlations for AD pre-exercise after $NaHCO_3$ ingestion and RPE_L after 1 minute ($\rho = 0.43$, $P = 0.003$, Figure 8.3) and 2 minutes during T_{LIM} ($\rho = 0.40$, $P = 0.005$, Figure 8.4).

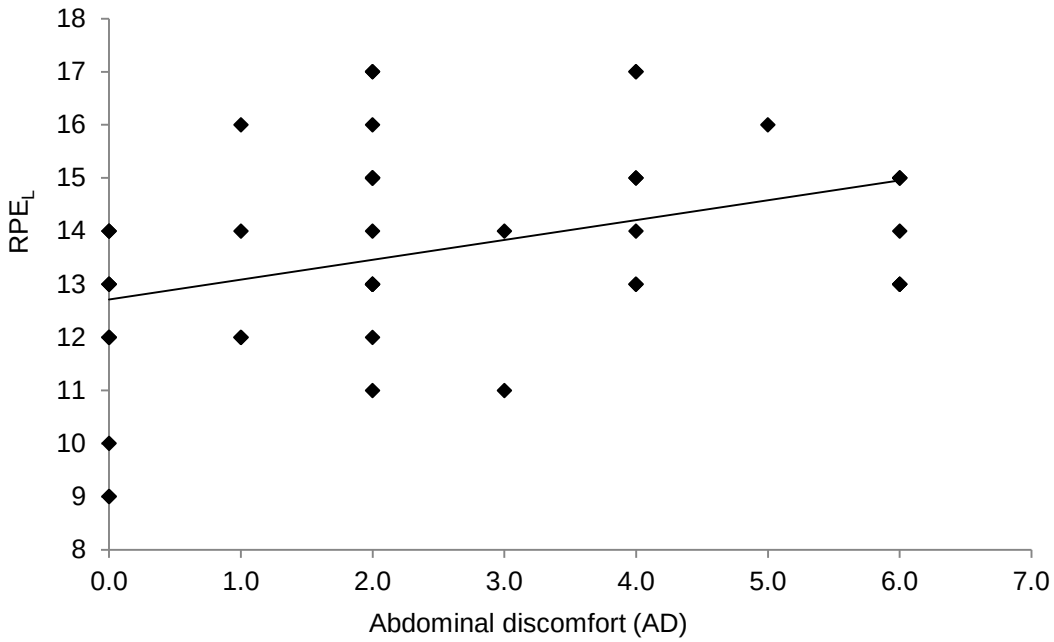

Figure 8.3 Correlation between RPE_L and pre-exercise abdominal discomfort (AD) after $NaHCO_3$ ingestion after 1 minute during T_{LIM} (n=18)

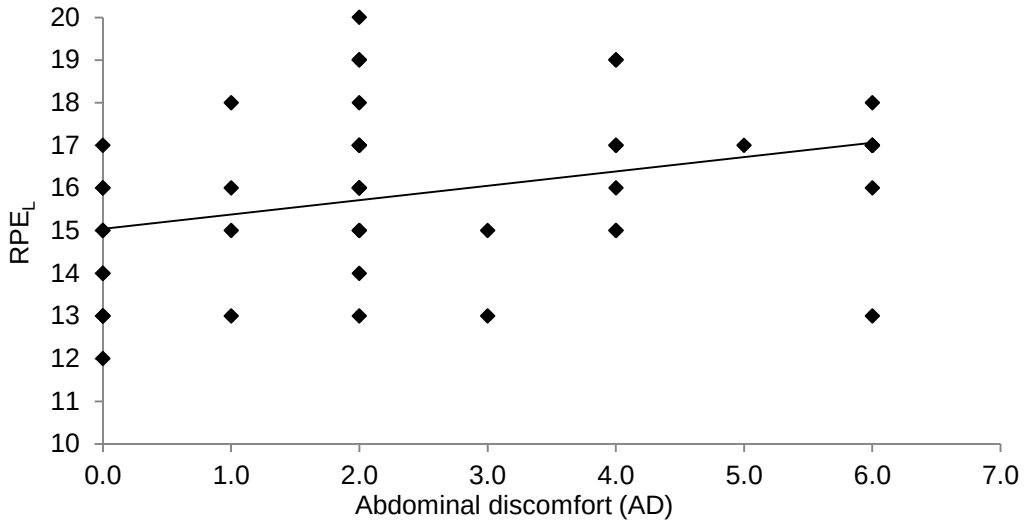

Figure 8.4 Correlation between RPE_L and pre-exercise abdominal discomfort (AD) after $NaHCO_3$ ingestion after 2 minutes during T_{LIM} (n=18)

The correlation between AD 30 mins pre-exercise and RPE_L after 2 minutes was low but approached significance ($\rho = 0.29$, $P = 0.052$). As $NaHCO_3$ has been shown to attenuate RPE_L during the early stages of T_{LIM} at 100% W_{PEAK} (chapter 5, 7) and that AD can negatively impact T_{LIM} (chapter 7) it's plausible that AD might actually 'dampen' the attenuation of RPE_L and subsequently negatively affect T_{LIM}. More research in this area is warranted.

8.3.4 Perceived readiness to exercise (PRE)

Overall (n=18), there was no relationship between PRE and AD or GF 30 mins pre-exercise or pre-exercise. Values of ρ ranged from 0.05 to 0.24 (P values: 0.11 to 0.75). Therefore, at a group level AD and GF appeared to have no effect on PRE. However, anecdotal feedback suggests that if participants report high levels of GI discomfort, because this is what they associate with $NaHCO_3$ ingestion they become, consciously or subconsciously, more aware of the possible benefits of $NaHCO_3$ ingestion. More simply, if

they have GI discomfort, many presume they have ingested $NaHCO_3$. As such participants might become 'more' motivated (or physiologically able) to tolerate the discomfort associated with T_{LIM} trials during $NaHCO_3$ trials compared to PLA trials. Interestingly, PRE for the participant who vomited ~ 30 mins after $NaHCO_3$ ingestion reduced from 9.0 pre-ingestion to 3.0 pre-exercise. However, as previously mentioned T_{LIM} improved by 11% compared to PLA. A similar trend was observed in this participant pre-training (although no vomiting occurred) in that PRE dropped from 8.0 pre-ingestion to 4.0 pre-exercise yet T_{LIM} improved by 21% compared to PLA. Changes in PRE due to time can be discounted as PRE remained high (8.0 to 9.0) at all time points prior to PLA trials for this participant. Therefore, despite suffering GI discomfort and an associated large reduction in PRE, T_{LIM} cycling performance, at an individual level at least, can still improve with $NaHCO_3$ ingestion. The mechanism(s) for this are yet to be elucidated but an increase in subconscious motivation based on GI symptoms (i.e. placebo effect), despite reported lower PRE, can't be discounted. As demonstrated in study 3 (chapter 6) this improved performance might be facilitated by augmented PO / capacity for work after $NaHCO_3$ ingestion.

8.4 What does the isolated muscle data tell us?

The isolated muscle model has allowed us to examine the effects of $NaHCO_3$ on skeletal muscle exercise capacity and acute PO without the possible influence of central fatigue, differences in motivation or mood and the inherent complexity (i.e. interaction of mixed muscle groups, complex muscle activation patterns) that constitutes locomotion. The data from this approach has provided important supporting mechanistic evidence for the acute ergogenic effect (i.e. increased acute PO) observed in humans (McNaughton 1992a, McNaughton, Ford, and Newbold 1997). The increases in acute PO in isolated skeletal muscle were due to greater force production throughout shortening. Similar to the variation in whole body data presented in study 2 (chapter 5) for T_{LIM} at 110% and 120% $W_{PEAK,}$ the fatigability of isolated muscle performance was variable with $NaHCO_3$. Therefore, in contrast

to whole body exercise performance, this suggests that at an isolated muscle level the 'responders', 'non-responders' classification might be appropriate. Importantly, regardless of treatment isolated muscles recovered almost completely within 60 minutes demonstrating fatigue rather than damage was the main reason for performance decreases during the fatigue protocol. Therefore, in those isolated muscles whose fatigability was improved with $NaHCO_3$, this did not appear to increase muscle damage (Figure 6.5).

8.5 Future work

The work presented in this thesis has examined the effects of $NaHCO_3$ on whole body and isolated skeletal muscle performance. As described in section 8.1 we have presented several novel findings that add to this body of research. However, in light of these findings a number of additional questions have been raised which warrant further examination. They are as follows:

1. We demonstrated that $NaHCO_3$ ingestion attenuates RPE_L after 2 mins exercise compared to PLA (chapters 5 and 7) although this was not observed after an improvement in training status (chapter 7). Further work should consider the possible mechanisms for why $NaHCO_3$ ingestion attenuates RPE_L after 2 mins exercise. An explanatory mechanism is yet to be fully elucidated but might be linked to the attenuation of perceived exertion by endogenous opioids (Sgherza et al. 2002) and/or by the rate of change in pH during initial stages of exercise (Lavender and Bird 1989, Price, Moss, and Rance 2003). Further research should also consider why an improvement in training status changes the attenuation of RPE_L in untrained males and whether AD dampens attenuation of RPE_L.

2. We evaluated the effects of $NaHCO_3$ on maximally stimulated mouse skeletal muscle (chapter 6). However, in order to address the efficacy of $NaHCO_3$ on exercise of longer

duration, further work should evaluate the effects of $NaHCO_3$ on submaximally stimulated mouse skeletal muscle. This approach has been considered in other ergogenic aids often used by humans to enhance physical performance (Tallis et al. 2012). Furthermore, in order to complement the human training study it would be beneficial to examine the efficacy of $NaHCO_3$ after an improvement in training status at an isolated muscle level.

3. We have provided exploratory evidence for a possible absolute threshold (rather than by body mass) of acute $NaHCO_3$ ingestion that optimises the chance of T_{LIM} improvement whilst minimising GI distress (Table 7.2). Based on this data and analysis of overall data (section 8.3.2) this appears to be ~ 23g $NaHCO_3$. Further research should establish whether such a threshold exists and how/if this might be adjusted based on a person's somatotype. One particular area of focus could be to examine the effects of $NaHCO_3$ on performance based on the operational muscle mass of a specific sport. For example, research should be carried out using cycling that evaluates whether acute $NaHCO_3$ ingestion modelled on lower body muscle mass is more beneficial than total body mass and whether an optimal maximum dosage exists.

4. Future work should consider whether an improvement in T_{LIM} after $NaHCO_3$ ingestion despite a reduction in PRE and concomitant increase in GI distress is repeatable, and if so what are the likely mechanisms that facilitate performance improvement in these circumstances. The possible effects of psychological factors such as intrinsic motivation and goal orientation (section 8.6.2) require consideration. Such further work should also incorporate different populations (i.e. untrained females, trained males and females) to establish whether such responses are population(s) specific.

5. More research is required to elucidate the mechanisms underpinning why an improvement in training status changes the efficacy of $NaHCO_3$. Based on the research

to date and the results presented in this thesis, training induced changes in intracellular buffering capacity (e.g. carnosine, Suzuki et al. 2004), training induced up-regulation of MCT1 and MCT4 (Pilegaard et al. 1999, Thomas et al. 2007) and possibly greater attenuation of the HSP72 response (Peart et al. 2011) are areas that require further research. Research utilising the muscle biopsy technique would appear to be fundamental in furthering this particular area of research. It would also be worthwhile evaluating the minimum training improvement required to alter the efficacy of $NaHCO_3$ as this might help to elucidate the mechanisms behind such a change in efficacy.

6. Due to the well reported possible negative GI side effects of $NaHCO_3$ ingestion, further research is warranted on other compounds that increase bioavailability of $[HCO_3^-]$ without such side effects. Recent research has demonstrated that ingestion of calcium lactate increased $[HCO_3^-]$ compared to CON and PLA. Subsequently, T_{LIM} and total work done at 100% W_{MAX} were 20% and 17% greater, respectively, than CON and PLA combined, the latter two not differing from each other. Moreover, such improvements were observed with no GI distress (Morris et al. 2011). Similarly, Heil, Jacobson, and Howe (2012) demonstrated that a new proprietary blend of ingredients called 'Alka-Myte' which purports to increase buffering capacity generated 3.1% greater PO compared to PLA against respective pre-intervention baseline trials during 60 s of upper body ergometry. This improvement in PO was reported with very minimal side effects, none of which were reported to have negatively affected participants.

7. Currently, there appears to be a lack of research examining the effects of $NaHCO_3$ on exercise performance with respect to the possible role of the brain/central nervous system. For example, Nakashima et al. (1996) demonstrated that infusion of $NaHCO_3$ increased cerebral blood flow, possibly from arterial dilation in response to CO_2, and Parham and Pasieka (1996) found that adding $NaHCO_3$ to lidocaine (widely used anaesthetic) reduced the associated pain of injection by ~ 27% against lidocaine only.

Although neither of these studies evaluated exercise performance we believe this demonstrates value in further examining how, if at all, $NaHCO_3$ might influence exercise performance from a neuro-physiological respective. Indeed, although some research has been completed (Hunter et al. 2009) Wu et al. (2009) agree that more research is required on the effects of alkalosis on neuromuscular function.

8.6 Limitations

8.6.1. General limitations

Perhaps the greatest limitation of the research within this thesis is that we have considered exercise induced fatigue largely from a metabolite accumulation perspective. Skeletal muscle fatigue as a result of high-intensity exercise is extremely complex and multi-factorial (Artioli et al. 2010, Debold 2012). Therefore, although clearly novel and relevant research findings have been made from both *in vivo* and *in vitro* models these should always be considered to be part of a far greater and more complex model than in isolation. More simply, factors outside of the possible physiological and biochemical contributors to fatigue should be considered. For example psychological factors such as intrinsic motivation, anxiety and mood can have a significant impact on fatigue and perception of fatigue.

8.6.2. Experimental limitations

Although great care and attention has gone into this research, as always there are some experimental limitations that have been observed in hindsight. They are as follows:

1. Although the Likert scales (or very similar versions) used for AD, GF and PRE (chapters 5 and 7) have been used in previous research (Price, Moss, and Rance, 2003, Nurmekivi et al. 2001, Price and Cripps 2012) the reliability and validity of these scales have not been experimentally determined. In particular the PRE scale used in this thesis was

adapted from a similar scale originally used in middle distance running (Nurmekivi et al. 2001). Therefore, it is plausible that a scale specifically designed to evaluate perceived readiness for high-intensity exercise and/or a scale that evaluates perceived readiness to exercise pre and post exercise after a nutritional intervention might be more suitable.

2. In study 2 (chapter 5), participants completed the achievement goal questionnaire (AGQ; Conroy, Elliot, and Hofer 2003) at the end of the study. Although this data still provides a useful understanding of goal orientated approaches that participants might use it would have been worthwhile to have issued the AGQ prior to experimental trials and mid-way through experimental trials. This would have allowed us to gain a more reliable view of participants goal orientated approaches and also to evaluate whether these changed throughout the study. Similarly, using the AGQ during the training study might have been useful to examine whether goal orientated approaches changed pre and post-training (especially considering the 'competition' that was promoted during training sessions) and whether this had any impact on the efficacy of $NaHCO_3$.

8.7 Practical implications

There are a number of practical implications that can be derived from this thesis. They are as follows:

1. Moderately but non cycling specific trained individuals are more likely to benefit from $NaHCO_3$ ingestion for high-intensity events and/or training that last between 5 to 10 minutes rather than 1 to 7 minutes as previously proposed. Specific events might include 5 km time trials in cycling or 2 km rowing races. However, as seen below this might also depend on an individual's muscle fibre type distribution.

2. As it is often impractical to analyse an individual's muscle fibre type distribution, individuals might benefit from understanding their somatotype and how this might influence exercise performance after ingestion of $NaHCO_3$. For example, mesomorphs who generally possess a greater proportion of (predominantly) type II fibres (FT), such as in EDL, might be more likely to see ergogenic benefit with $NaHCO_3$ during high-intensity exercise of short duration (i.e. ~ 1 to 4 minutes) where the ability to produce high acute PO is likely to be important to performance. However, ectomorphs who generally possess a greater proportion of ST fibres, such as in SOL, might be more likely to see ergogenic benefit with $NaHCO_3$ in events of longer duration.

3. Although GI distress after $NaHCO_3$ ingestion is uncomfortable, those who suffer such symptoms should not assume that exercise capacity will always be detrimentally affected, even if self perception of readiness to exercise reduces. Therefore, where $NaHCO_3$ ingestion is incorporated into physical training, individuals and coaches alike should base decisions largely on performance data rather than anecdotal feedback.

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Chapter 10 – Appendices

10.1 Abdominal discomfort (AD) Likert scale

0 Completely comfortable

1

2 Fairly comfortable

3

4 Slight discomfort

5

6 Moderately discomfort

7

8 Extreme discomfort

9

10 Unbearable pain

10.2 Gut fullness (GF) Likert scale

0 Empty

1

2 Slightly Full

3

4 Fairly Full

5

6 Moderately Full

7

8 Uncomfortably Full

9

10 Bloated

10.3 Perceived readiness to exercise (PRE) Likert scale

10	**Completely ready to exercise**
9	
8	**Largely ready to exercise**
7	
6	**Moderately ready to exercise**
5	
4	**Somewhat ready to exercise**
3	
2	**Hardly ready to exercise**
1	
0	**Not at all ready to exercise**